The Archives of Internal Medicine

Vol. XII NOVEMBER, 1913 No. 5

THE CAUSE OF THE SPECIFIC DYNAMIC ACTION OF PROTEIN *

GRAHAM LUSK

NEW YORK

On the occasion of the International Congress of Hygiene and Demography held in Washington in 1912, it was pointed out that the ingestion of 25 grams of glycocoll or of 20 grams of alanin, each of which is convertible in the organism into 20 grams of glucose, caused a very great rise in the quantity of heat produced by a dog, whereas the ingestion of 20 grams of glucose itself had a slight or a negligible influence. It was also shown that when glutamic acid was administered there was no increase in the heat production of the dog. Since glutamic acid yields both sugar and urea in the course of its metabolism, it was concluded that the increased heat production after giving glycocoll and alanin could not be due to the processes of sugar formation, of deamination or of formation or elimination of urea. Also, ingestion of urea gave negative results as regards increased heat production. It seemed, therefore, that the stimulus to greater heat production given by glycocoll and alanin must have been due to their own chemical individuality. It was further shown that leucin and tyrosin increased metabolism, and that a mixture of 5.5 grams of each of the five amino-acids, glycocoll, alanin, glutamic acid, leucin and tyrosin caused an effect on metabolism which was as pronounced as that observed after giving 25 grams of glycocoll alone. It was greater than that following the ingestion of 100 grams of meat which contained about the same quantity of nitrogen. That the effect of this mixture of the various amino-acids was one of summation is shown in the following new experiments.

The experiments were performed on Dog 3, weighing 12 kilograms, whose basal metabolism (that obtained eighteen hours after administering a standard maintenance diet) as determined on February 26, 27

* Address delivered at the International Physiological Congress, Groningen, September, 1913.

* From the Physiological Laboratory of the Cornell University Medical College.

* Submitted for publication July 21, 1913.

and March 28, was found to average 19.8, 19.7 and 19.9 calories per hour. The amino-acids were given eighteen hours after the ingestion of food and the metabolism determined for three or four hours afterward. It was found in this way that the increase in heat production after giving 5.5 grams of glycocoll was 3.1 calories, after 5.5 grams of alanin it was 3.9 calories and after a mixture of 5.5 grams of each 8.5 calories. In other words, the basal metabolism was raised by glycocoll in this small amount 5.2 per cent., by alanin 6.6 per cent. and by the two mixed together 14.1 per cent. These experiments prove that there is a summation in the heat-increasing power when the two amino-acids are ingested together. This one would expect to find from the behavior of metabolism following meat ingestion.

The results of these experiments reinforced the conclusion already published that the increase in metabolism after the ingestion of meat is due to the mass action of absorbed amino-acids acting on cellular protoplasm.

An *experimentum crucis* was devised to determine whether glycocoll itself is a stimulus to metabolism. When glycocoll is administered in phlorhizin glycosuria its energy content is almost entirely eliminated in the form of sugar in the urine. It may be computed that 10 grams of glycocoll containing 31.10 calories of energy are converted into 8 grams of sugar with 29.52 calories and 4 grams of urea with 10.11 calories. The reaction is, therefore, endothermic. If the heat production be increased after giving glycocoll in phlorhizin glycosuria it would be certain that this would be due to the chemical nature of the substance and not to its energy content.

Considerable difficulty was occasioned by the fact that the fasting diabetic animal, when placed in the respiration calorimeter at a temperature of 26, almost invariably vomited the solution of glycocoll administered. After six experiments on four different dogs success was obtained. The record of metabolism began an hour and three quarters after the ingestion of 12.5 grams of glycocoll. As on other occasions, the dog manifested polypnea about an hour after administration of the glycocoll. On this occasion the animal was held in an upright position for over an hour before placing in the calorimeter, a procedure which often prevents vomiting. That the cause of the polypnea is due to the increased production of heat may be deduced from experiments of Wiggers, who introduced 4 c.c. of N/10 solution of glycocoll into the vein of an anesthetized cat without changing the nature of the respiration.

The *experimentum crucis* showed that the effect of glycocoll was to increase the fasting phlorhizin metabolism of 25.9 calories per hour to one of 31.4 calories, a rise of 21 per cent. A calculation from the urinary findings showed that within the short period of four hours an elimination

of 7 of the 10 grams of extra sugar which can arise from 12.5 grams of glycocoll, actually occurred. From former experiments there can be no doubt that essentially all the glycocoll was converted into sugar. A control experiment showed that the ingestion of 10 grams of glucose did not cause an increase in heat production, although 4.7 grams of extra glucose were eliminated in the urine during the period of three hours. Since the potential energy of 12.5 grams of glycocoll is largely eliminated in 10 grams of urinary sugar, and the actual elimination of sugar is not accompanied by increased heat production, it follows that the rise in metabolism after glycocoll ingestion is not derived from the energy content of the glycocoll administered, but that glycocoll itself acts directly as a stimulus to metabolism.

There is only one other possibility, namely, that advanced by F. G. Benedict, to the effect that organic acids are the effective stimuli. The chief support of Benedict's hypothesis lies in his statement that d-fructose increases the heat production in diabetics even though much of this sugar be converted into glucose, and none of it is oxidized. Here one might surmise that such intermediary products of sugar metabolism as lactic acid or methyl glyoxal (Dakin) might be the effective stimuli to increased heat production. In like manner, the oxy-acids glycollic or lactic, derived from glycocoll or alanin, or the keto-acids of the same series might be the effective stimuli after the ingestion of the amino-acids.

To investigate this problem, ethyl glycolate and ethyl lactate have been administered to dogs. When the former was given in doses of 12 grams it proved to be a toxic agent. Ethyl lactate, however, increased metabolism, and to a much higher extent than did its alcohol component given alone. Lactic acid itself has not been given, and experiments with glycollic acid have proved futile since it was vomited whenever administered. The work with ethyl lactate does not necessarily prove that lactic acid is in itself a stimulus to metabolism, as the effect produced might have been due to the action of some of the absorbed ester. The affirmation of Benedict's theory cannot, therefore, be given.

Pending further investigation it is well to recall that glutamic acid, which is a dicarboxyl amino-acid, exerts no influence whatever on heat production, although this substance forms urea and is in part synthesized into sugar in the body. This behavior favors the validity of the statement made a year ago that the increase in metabolism which follows the ingestion of meat is due to the mass action of amino-acids acting on cellular protoplasm.

First Avenue and Twenty-Eighth Street.

REFERENCES

Lusk: Jour. Biol. Chem., 1912, xiii, 155.
Benedict: Deutsch. Arch. f. klin. Med., 1913, cx, 154.

SOURCES OF ERROR IN THE USE OF THE STOMACH-TUBE FOR DIAGNOSIS. PRELIMINARY REPORT *

TORR WAGNER HARMER, M.D., AND WALTER J. DODD, M.D.
BOSTON, MASS.

It has been our experience that the unaided stomach-tube occasionally fails to detect gastric stasis which is demonstrable with the bismuth meal and Roentgen ray. Discrepancies in the Roentgen ray and stomach-tube findings were apparently not due to the position of the patient at the time of the passage of the tube; for we have observed them with patients in the sitting, supine and right reclining positions. The long axis of the stomach is more perpendicular than horizontal, but inclines somewhat to the right. Theoretically then, when the patient is reclining slightly on the right side, the gastric contents should occupy a narrower portion of the stomach than when the patient is in the erect sitting position, which is commonly employed in using the stomach-tube. The contents of the stomach in the slightly reclining right-sided posture should be of greater depth than when more spread over the greater curvature, as is the case in the erect sitting posture, and should, therefore, be more readily siphoned off with the tube. Even with the patient in this position we have failed with the tube to detect residuum which was demonstrable with the Roentgen ray. Curious to determine the cause of this inefficiency of the stomach-tube, we were led to watch by means of the fluoroscope, the course taken by the tube. We have observed in several individuals that the end of the tube did not pass directly to the most dependent portion of the stomach. In these cases, the tip of the tube first touched a portion of the stomach wall well above the most dependent portion. As more tube was passed, the end of the tube slid along the wall of the stomach a greater or lesser distance and became impinged against the stomach wall. On further passage of the tube, the end remained fixed and a downward bowing of the tube into the lower portion of the stomach occurred. This downward bowing of the tube apparently diverted the direction of force on the tip of the tube; for, as more tube was passed and the dependent loop of tube increased in size, the tip sooner or later slipped from its fixed position. The force on the tip was then upward through the dependent loop of tube and consequently the tip slipped upward toward the cardia; that is, it became further removed from any residuum which may have been present in the stomach. On passing still more tube, the end may follow about the wall of the stomach

* Submitted for publication, Aug. 18, 1913.

and may eventually reach its most dependent portion. We have observed, however, that it may again curve horizontally and then upward. The tip of the tube may skim the surface of the residuum and therefore drain only a portion of it. Further passage of the tube in the hope of reaching a deeper level of the contents may fail, because the tip may curve upward. The result in such cases may not be seriously deceptive. The degree of

Figure 1

stasis may be underestimated, but the analysis of the siphoned contents may show stasis. In some instances, however, considerable amounts of residuum may entirely escape detection by the tube. Although the accompanying skiagraphs do not show the course of the tube as satisfactorily as the fluoroscope, they are sufficiently indicative and require but little explanation.

Figures 1, 2 and 3 illustrate a successful passage of a stomach-tube. Figure 1 shows the outline of the stomach with bismuth meal. The lower border of the stomach is on a level with the inferior surface of the fourth lumbar vertebra. The patient is in a marked inclined position. In Figure 2, measuring from the incisor teeth, 17½ inches of the tube

Figure 2

have been inserted. The end of the tube is shown opposite the lower surface of the first lumbar vertebra and the bismuth residue opposite the middle of the fourth lumbar vertebra (just below the umbilicus, as indicated by the circular marker). Further passage of the tube was observed on the fluoroscopic screen and when the tip of the tube reached the bismuth residue, Figure 3 was taken. Twenty-six and one-half

inches of tube had then been passed, or 3½ inches beyond the tooth-mark on this tube.

Figures 4 and 5 illustrate the course of the tube in another case, an unsuccessful passage. The pictures were taken immediately after the

Figure 3

ingestion of a bismuth meal with a considerable amount of fluid. The patient is standing. There is no gastroptosis, the lower border of the stomach being just above the umbilicus, as indicated by the marker. As the tip of tube emerged from the esophagus into the stomach, the fluoroscope showed that it curved almost immediately to the left and became impinged against the stomach wall. On the passage of more tube a dependent loop formed. When 23 inches of tube had been passed, Figure

Figure 4

4 was taken. The fluoroscopic observations in the meantime showed the tip had remained practically stationary. The figure shows the tip of the tube an inch above the level of the fluid in the stomach. Even the lowest portion of the tube (the bottom of the dependent loop) is only at the top of the bismuth meal, that is, 2½ inches above the most dependent portion of the stomach. In Figure 5, 16 inches more of the tube have

been inserted, a total of 39 inches. It is seen, however, that the tip of the tube occupies practically the same position as in the previous figure. The tip is apparently firmly impinged. The dependent loop has increased in size and has advanced into the pyloric end of the stomach. Owing to the narrowness of this part of the stomach, the curve of the loop has

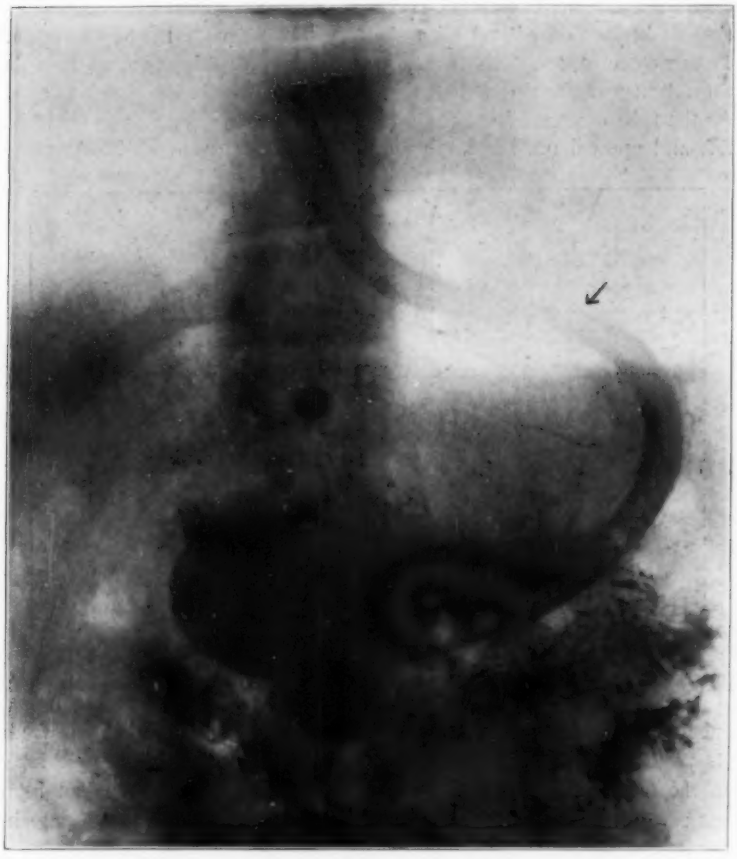


Figure 5

become sharper. The formation of this loop, as seen on the fluoroscopic screen, is shown in the accompanying diagrams (Fig. 6). Diagram (a) corresponds to Figure 4. Diagram (g) corresponds to Figure 5. Up to this point, the passage of 39 inches of tube, no fluid was siphoned from the stomach. The explanation is obvious from the figures, which show

that the tip of the tube was constantly above the level of the fluid. The tube was then gradually withdrawn, but still no fluid was siphoned off. The tip at no time receded beneath the level of the fluid, merely the loop was withdrawn.

Figures 7, 8 and 9 illustrate another unsuccessful passage of a stomach-tube. Figure 7 shows the size, shape and position of the stomach, the lower border being just below the umbilicus, as indicated by the marker, or on a level with the top of the fourth lumbar vertebra. Fluoroscopic observations revealed a very similar condition, as regards the relation of the tip of the tube to the cardia, as in the previous case. As the tube emerged from the esophagus, it curved directly toward the left and upward until it impinged against the stomach wall. As more

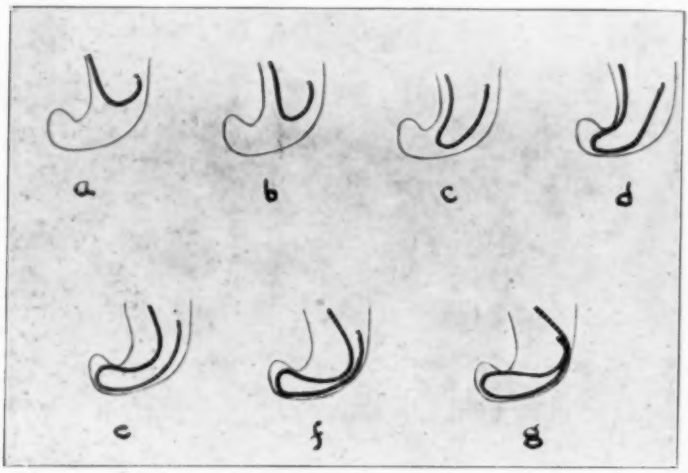


Figure 6

tube was passed a dependent loop formed. The tip finally slipped from its position against the stomach wall and came to lie at a higher level in the cardia. Figure 8 was taken when 35 inches of tube had been passed. The tip is in the magenblase, as indicated by the light space in the figure. The lowest part of the loop is just below the top of the third lumbar vertebra, or about 1 inch above the lowest portion of the stomach (see Fig. 7). In an effort to reach the lowest portion of the stomach with the tip of the tube, more and more tube was passed and constantly observed on the fluoroscopic screen. When 46 inches tube had been inserted, Figure 9 was taken. At no time during these fluoroscopic observations did the tip or any other part of the tube reach the lowest portion of the stomach. The figure shows the lowest portion of the tube

at the level of the middle of the third lumbar vertebra or about 1 inch above the lowest border of the stomach (see Fig. 7). The tip of the tube is on a level with the top of the second lumbar vertebra. The accompanying diagrams (Fig. 10) illustrate the course of the tube during its com-

Figure 7

plete passage as shown on the screen. Diagram (c) corresponds to Figure 8. Diagram (g) corresponds to Figure 9. It will be seen that the dependent loop of tube, in this case, descended into the lower portion

of the stomach and that the passage of more tube caused the formation of several kinks. At no time did the tip reach the lowest portion of the stomach, and, furthermore, the kinks themselves would have prevented the siphonage of any residue.

Figure 8

Curious to ascertain why the tip of the tube may become deflected in the cardia on emerging from the esophagus instead of passing directly to the most dependent portion of the stomach, we were led to pass stomach-tubes through a section of glass tubing about the length and diameter of the esophagus. We have frequently seen the tip of the stomach-tube, after emerging from the lower end of the glass, deflect

from the long axis of the glass. Stomach-tubes are delivered from the manufacturers coiled. Stomach-tubes are commonly kept coiled. Just before passing they are commonly coiled in basins or pitchers of ice or cold water. The tube acquires a tendency to curve, and we believe it is

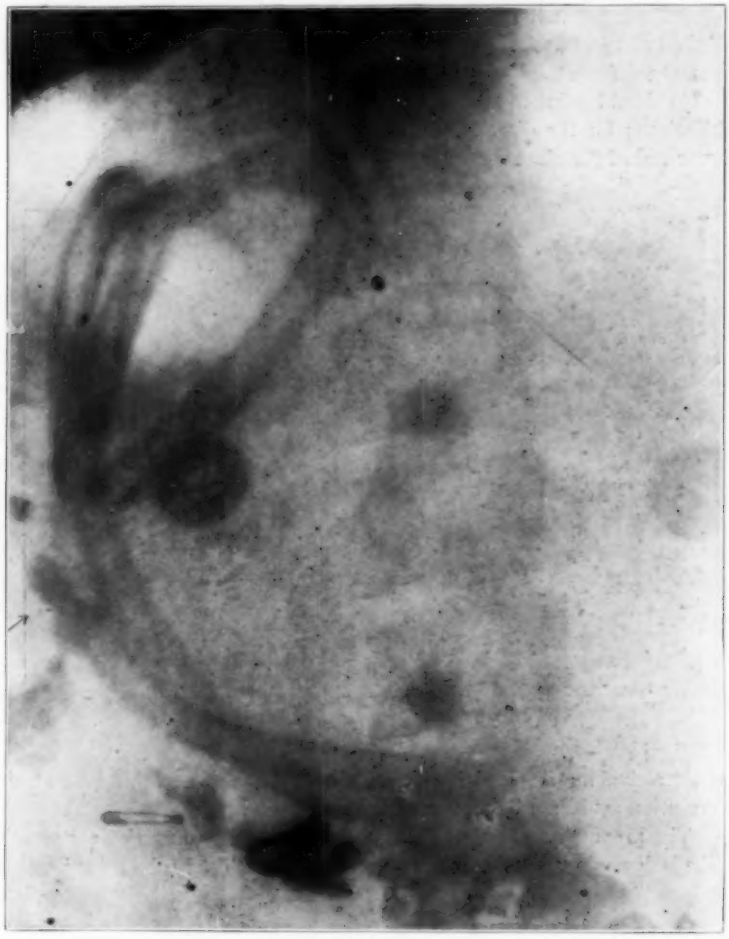


Figure 9

the tendency so acquired which is responsible for its deviation from a straight course after emerging from the lower end of the esophagus. If the curve of the tube accommodates itself to the curves of the stomach wall and if the tip does not become impinged against the stomach wall,

the tip will reach the most dependent portion of the stomach. Deductions will be correct. If, however, the curve of the tube does not accommodate itself to the curves of the stomach wall and the tip becomes impinged against the stomach wall, the tip may not reach the most dependent portion of the stomach. Deductions will be incorrect.

Believing that it might be possible to allow for the curving tendency of the tube, we have started to pass the tube in such a position that, when it emerged from the lower end of a glass tube representing the esophagus, its tip should point toward the pylorus. This reasoning was shown to be fallacious; for the direction of the tip at times as it emerged from the lower end of the glass was contrary to expectations. The tube, in passing,

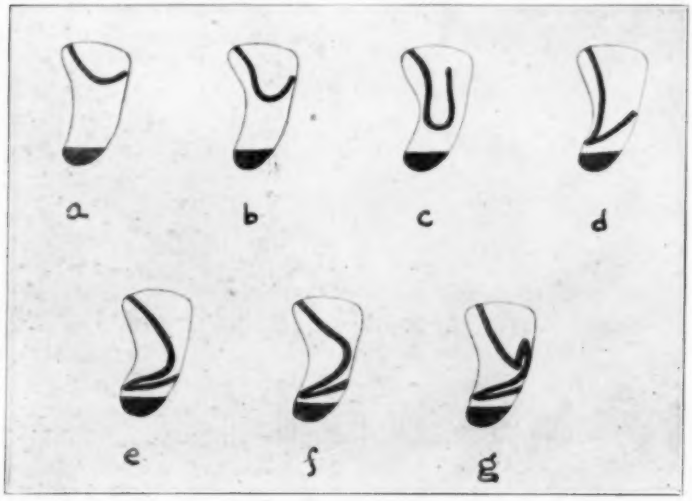


Figure 10

at times became revolved by its contact with the glass tubing, and this revolution could not be prevented with certainty.

The proportion of successful and unsuccessful passages of a stomach tube is dependent in part on the tightness in which the tube is kept coiled and on the flexibility of the tube. The more flexible is the tube, the less apt is it to maintain a curve after coiling, but the more apt is it to kink when its tip impinges against the stomach wall. The stiffer is the tube, the more apt is it to maintain a curve after coiling, but the more apt is its tip to slip along the stomach wall.

The proportion of successes and failures is also dependent on the posture of the patient and on the position the stomach occupies in the abdominal cavity. In cases of gastroptosis the degree of displacement

may be underestimated and an insufficient length of tube passed. This, we believe, is a common error. Contrary to our expectations, however, we have found in some cases of even marked ptosis that, if enough tube be passed, the most dependent portion of the stomach may be reached

Figure 11

despite the tendency of the tube to curve. In such cases it appears that a long, tubular cardia tends to guide the tube to the bottom of the stomach. Figures 11, 12 and 13 illustrate such a case.

Figure 11 was taken immediately after the ingestion of a full bismuth meal and shows the lower border of a long, tubular stomach 1 inch above

the symphysis pubis. In Figure 12, 23 inches of tube (that is, to the tooth-mark) have been passed. The tube is shown inclining toward the left border of the stomach, the tip opposite the top of the fourth lumbar

Figure 12

vertebra. During the passage of this tube, as in previous cases, fluoroscopic observations were constantly made and the tip of the tube was seen to enter the bismuth meal. Figure 13 was taken when 34 inches of tube had been inserted. The tip has emerged from the bismuth residue

Figure 13

and is on a level with the middle of the fourth lumbar vertebra. This case illustrates a successful passage of a tube and apparently the long, tubular form of the cardia and perhaps the conformity of the false pelvis tended to direct the tip to the most dependent portion of the stomach. In this case the passage of 31 inches of tube was necessary to reach the

lowest part of the stomach (that is, 8 inches beyond the tooth-mark on the tube used). Several facts were shown in this case. If exactly 31 inches of tube had been inserted, the bottom of the fluid would have been reached, siphonage would have been complete and deductions as to the amount of residuum correct. But as there is no way of knowing exactly when the lowest level of the residuum is reached, it is obvious that too much or too little tube may be passed. In this case, if somewhat less than 31 inches of tube had been passed, siphonage might have been established, but the passage of more tube on the cessation of flow, in the hope of reaching the lowest level of the residue, might have failed, owing to the passage of too much tube, the tip emerging above the fluid level. Under these conditions, the gradual withdrawal of tube in the hope of resubmerging the tip would not reestablish siphonage once broken. This fact may be easily demonstrated with a tube and a partially filled flask.

Further observations are being made on various types of stomach and experiments in the construction of a tube which will obviate these difficulties.

From these simple observations it is obvious that failure to recover gastric residuum with the unaided stomach tube from a fasting stomach or after the ingestion of a test meal cannot be accepted as conclusive evidence of the absence of gastric stasis.

192 Marlborough Street—205 Beacon Street.

THROMBOSIS *

LUDWIG ASCHOFF

FREIBURG, I. BR.

In the year 1911, at the meeting of the *Deutsche Naturforscher und Aerzte,* I submitted a review on the subject of Thrombosis. The question was brought up because the increasing number of operations in surgical and gynecological practice has made a fatal embolism of the lung, the sequel of a thrombosis, of more and more frequent occurrence.

The more we try to obviate this dangerous complication, the more urgent is our obligation to find out the cause of thrombosis. Now, there is no doubt at all, to use a mathematical figure of speech, that thrombosis is the function of a number of variables. There is not a *single* cause, but quite a number of different conditions which are closely related to the occurrence of thrombosis. Among these may be mentioned here, first, changes in the blood-plasma (diminished or increased coagulability); secondly, changes in the blood elements (increased or diminished powers of agglutination); thirdly, changes in the blood flow (slowing and formation of eddies), and lastly, changes in the vessel wall itself (endothelial damage). An inquiry into the mechanism of thrombosis shows that sometimes one factor, sometimes another, plays the principal rôle.

The view that increased coagulability of the blood is an essential point for the production of thrombosis, has been strongly upheld, especially by clinical observers. The existence of this increased coagulability, and the likelihood that it is a promoting factor, or, better, an accompanying phenomenon of thrombosis, cannot be denied. But all histological research since the early work of Zahn, Eberth and Schimmelbusch, Welch and others, speaks for the view that in human beings the occurrence of fibrin coagulation is not the first stage of thrombosis, but that important changes in the morphological blood constituents precede it. These latter changes must be explained before the mechanism of thrombosis can be understood.

It is concerning the morphological structure of a thrombus, in continuation and amplification of my review mentioned above, that I wish to speak.

MORPHOLOGICAL STRUCTURE OF THROMBI

Since we have known from the work of Zahn, on the one hand, and of Eberth and Schimmelbusch on the other, that the blood-platelets and leukocytes really have to do with the building of a thrombus, the idea

* The Cartwright Lectures for the year 1913, of the Association of the Alumni of the College of Physicians and Surgeons, Columbia University.

readily suggested itself that the whiter color of one part of a thrombus was to be referred to an accumulation of these elements, which would thus appear to constitute the first material laid down in the process. We have become accustomed to speak of white, mixed and red thrombi, but the relationship of these different appearances to one another, in spite of the early work of Zahn and others, has not been wholly and correctly appreciated in the literature of the subject. We must remember with Welch—to whom we must give thanks, as well as to the previously mentioned authors, for the most exhaustive experimental work on thrombosis—that in a completely finished thrombus, for example in the femoral vein, the variations in the colors are perfectly regular. The first part (*Kopfteil*) of the thrombus is chiefly of a white color, and represents the so-called white thrombus, and on this a middle part (*Halsteil*), mixed in color, and a deep red, distal and final portion (*Schwanzteil*) are subsequently laid down. The first part (*Kopfteil*) may be of the smallest possible size and extent; whereas the final red part (*Schwanzteil*) often forms the bulk of the thrombus, and may measure many centimeters. Thrombi in other parts of the venous system are built up on exactly the same principle.

It is easy to understand that red thrombi occur only where a white thrombus has more or less completely obstructed a vessel, and that they cannot occur where the white thrombus only partially obstructs the lumen.

Very long white thrombi can arise only when the blood-stream continues to pass through a vessel. From all these points a rule can be formulated which holds for the majority of all autochthonous thrombi in human beings; namely, that the white thrombus is the determining and peculiar factor in the whole process, and that red thrombi are only, so to say, accidental—they may occur but not necessarily. An exception would of course be the case of a pure red thrombus arising by autochthonous thrombosis, and not consisting of a piece of an ordinary thrombus which had been broken off and carried away. Such an event, if it occurs at all, is certainly very rare.

Our interest, then, centers on the question of what this *Kopfteil*, or first part of a thrombus, looks like under the microscope. The structure of it will perhaps give us an understanding of its mode of origin.

First of all, we must remember that the much quoted Zahn's markings on the outer surface of thrombi—the small ripple-like, net-like and linear markings — are only to be seen on the *Kopf* and *Halsteil* of thrombi, whereas in the red part of the thrombus they fade and soon totally disappear. These markings are merely fine, white elevated lines or ridges which are specially well brought out when the furrows between are reddish in color, or where a mixed red and white thrombus, as in the *Halsteil*, is present. Along with the explanation of this marking stands or falls the

whole problem of thrombus formation, so far as consideration of the majority of cases of autochthonous thrombosis goes. This decision appears at first sight a bold one, but will readily be understood when the microscopic structure has been taken into account. A longitudinal section through the *Kopf* and *Halsteil* of a thrombus shows that the very delicate surface elevation is only the summit of a framework of beams, which in delicate rings like a mass of coral, forms the skeleton of the whole thrombus. When a thrombus at quite an early stage in its development is examined, this framework is seen to be a finely granular mass which consists simply of an accumulation of blood-platelets. All the beams of this framework are surrounded by a border of polymorphonuclear leukocytes, by means of which they are differentiated even more sharply from the red blood-mass which fills up the numerous spaces between.

But it is not only the regular and definite separation of the different blood-elements which is surprising, but the further fact that all the beams of the framework stand in a very special relationship to one another. The beams follow one another at fairly regular intervals, and build up group-like systems, inside of which the direction of the beams is much the same. In addition, however, secondary beams are seen in these groups extending either upwards or downwards from the primary beam of the framework. It must be admitted that dissimilarities also occur here. The more we approach the pointed outer extremity of the *Kopfteil* the broader become the beams of the framework, the furrows between disappear, until finally the beams unite into a single mass, to form the pure white outer surface of the *Kopfteil*.

The most important point, however, in the structure of this whole system is that fibrin practically does not appear. When it is found, however, the strictest regularity governs its situation. The threads of fibrin shoot out first of all along the borders of the framework of blood-platelets just where the red blood touches the framework. Subsequently it penetrates more and more into the blood itself. A structure such as has just been described is the only sure token by which we can recognize microscopically the intravitam origin of a thrombus.

With this explanation of this structure, we are enabled at the same time to understand the way in which a thrombus is built up. The explanation involves the solution of a complicated physical problem, which I can present here only in a general way, since, in spite of many discussions with my colleagues in the department of physics, I have been unable to get to the bottom of it. What the vital question is, we can state nowadays with certainty. This question is: Does the thrombus arise in the flowing or in the stationary blood-stream? So long as the view was held that white thrombi were built up from leukocytes, as Zahn pointed out, it had to be assumed that it was only in the flowing blood

that these thrombotic masses could be laid down. Zahn himself laid great stress on this point. But when it became practically certain that a thrombus takes origin exclusively from blood-platelets, uncertainty again arose, since the origin and meaning of these platelets was veiled in obscurity. While some observers affirmed the independence of these structures, many more considered them to be merely distintegration products of white and especially of red corpuscles. If the latter theory were true, a thrombus could quite well be considered to be formed from a small mass of dead and disintegrated red blood-cells, which had died because of the stopping of the blood-stream. But it could not explain why the heap of particles at once proceed to build themselves up into such a beautiful framework and divide themselves off so sharply from the red corpuscles.

Taking it for granted that the origin of blood-platelets is not from leukocytes or erythrocytes, but that they are independent elements existing by themselves in the blood-streams (as indeed the excellent researches of Deetjen have already suggested), it becomes quite clear to us that such accumulations of blood-platelets as occur in a thrombus can be deposited only when the blood is circulating. The important question of the origin of the blood-platelets can, in my opinion, now be regarded as settled. They take origin neither from white nor red corpuscles, but are, for so long as they circulate in the blood, independent structures. J. H. Wright of Boston was the first to point out their origin from the giant cells of the bone-marrow and spleen. In his important researches he was able completely to confirm the statement of Schridde that the megakaryocytes have a finely granular protoplasm except for a zone at the margin, which remains clear. He then showed that from the giant cells small portions are nipped or budded off in the form of small platelets, each of which consists of a finely granular central portion surrounded by a clear marginal zone. These are in fact the blood-platelets, and the granular center, the origin of which is thus readily understood, is identical with the structure often described, especially by Deetjen, as a nuclear structure in the platelet. That independent movement is possessed by these structures had already been shown by Deetjen. Wright's findings, doubted on many sides, were reinvestigated by Ogata in the Pathological Institute in Freiburg, and were all completely confirmed. In fact in fresh, warm material taken from different animals for investigation the budding off of platelets from the megakaryocytes can be plainly followed. At the time of the budding-off process the protoplasm of the giant cell is seen to have invaginated itself into a capillary in the form of a pseudopod; and thus the occurrence of the platelets in the blood-stream comes about.

The independence of the blood-platelets in the circulation can be proved in another and very positive way. If they arose simply by the disintegration of red corpuscles, then the destruction of a stationary column of blood, as can readily be produced in a doubly ligated vessel by the corrosive action of a small piece of silver nitrate, would be followed by a blood-platelet thrombus-building, just as occurs in the flowing blood-stream where a typical thrombus forms on a damaged piece of vessel wall. This question was investigated by Derewenko[1] in the Pathological Institute in Freiburg with striking results. He found not a trace of heaping-up of platelets in the doubly ligated vessel in spite of all the damage, whereas in a vessel through which the blood still flowed a typical thrombus had been built up from blood-platelets, showing the characteristic framework we have learned to recognize in the human subject.

We have concluded then, that the thrombus arising from the blood-platelets has its origin in the circulating blood-stream, and the question yet to be answered is: Why do the platelets build themselves into this peculiar framework? To this important question I can give no final answer. A few interesting points only may be raised. By the making of serial sections of human thrombi, and subsequently building up models, Ferge,[2] working in the Pathological Institute in Freiburg, was able to determine with certainty that the platelets do not form beams running in circles but do form a system of lamellae, more or less parallel and arranged on the vessel wall one behind another. Most of them run obliquely or transversely to the long axis of the vessel. These lamellae grow outward from the vessel wall, and from the flow of the blood through a part of them, they become bent in the direction the blood is flowing. The appearance of this system is strikingly similar to the beautiful figures made on the fine sand of the seashore by the ebbing tide. The water pushes up the fine sand into ridges, until the strength of the flow overcomes the small obstacle, and the water travels on to build new ones further on. In this way then the beautiful series of ripple lines are drawn on the shore. The blood-stream builds up its lamellar system in an exactly similar way from the sand-like multitudes of blood-platelets. Zahn had previously taken notice of the work of de Candolle on the curious sand formations produced by moving water, and had put forward the suggestion that the lines visible on the surface of the thrombus are simply the effect of to and fro movements of waves of blood. But the essence of the matter is not that already existing sandy masses are shaped into systems by the movements of the blood, but that solid elements are taken from the flowing blood-stream and laid

1. Derewenko: Zieglers Beitr., 1910, xlviii, 123.
2. Ferge: Med.-naturw. Arch., 1909, ii, 351.

down in the form of specially situated lamellae. I can only mention here in passing that by experiments which I carried out in the laboratory of Professor Rehbock I was able to find exactly similar deposits formed in running water from a given solid material, when the velocity of the flow was reduced sufficiently by the introduction of a dam or weir. No transformation of already laid down masses of particles was necessary to make the ridges, but the particles were laid down in such a way from the first. The question, of course, arises from these experiments as to why a deposit of platelets occurs at all, and this will be discussed later on. So long as the blood flows through this framework just as sea water flows beneath branches of a tree of coral, new masses of blood-platelets are laid down on the system already formed, and so new systems are always being built up behind the first one. The first formed system naturally grew faster, and finally there comes a time when the openings in the lamellae of the old part of the thrombus (this is indeed the *Kopfteil* which has already frequently been referred to) are so narrowed that the blood-stream becomes slower and slower, and finally stops altogether. With this event the building of a primary thrombus is finished, since once the blood-stream is at a standstill no new blood-platelets can be carried past, and hence further growth is impossible.

But before the complete cessation of the blood-stream occurs, a certain peculiar separation of the red and white corpuscles comes to pass, resulting in the whole system of platelets becoming covered with a layer of leukocytes. The facts of this peculiar separation are best explained by the well-known researches of Eberth and Schimmelbusch, who found that when the blood-stream was slowed the white corpuscles being of lighter specific gravity than the others, tended to travel at the margin of the blood-stream, and so are found closely in contact with the vessel wall. By the growing system of platelets which occurs in the formation of a thrombus the main blood-stream is divided up into a series of small streamlets. Each small streamlet is, however, subject to the same laws as a larger vessel, and when the slowing of the blood-stream occurs, the leukocytes are found close to the sides of each small streamlet, whose wall is in this case composed of the framework of blood-platelets.

Before I can turn to the question of why the blood platelets are deposited from the circulating blood, I must briefly describe the results which follow in the peripheral part of the vessel, when the stream is completely interrupted through the gradually increasing obstruction of a system of platelets. As soon as the lumen is closed in the region of the white, that is the primary thrombus, the whole blood-column becomes stationary right up to the place where the next anastomosing vessels enter, and undergoes very rapidly, as experiment shows, a complete coagulation. In this *Schwanzteil* of a thrombus, which is usually of some

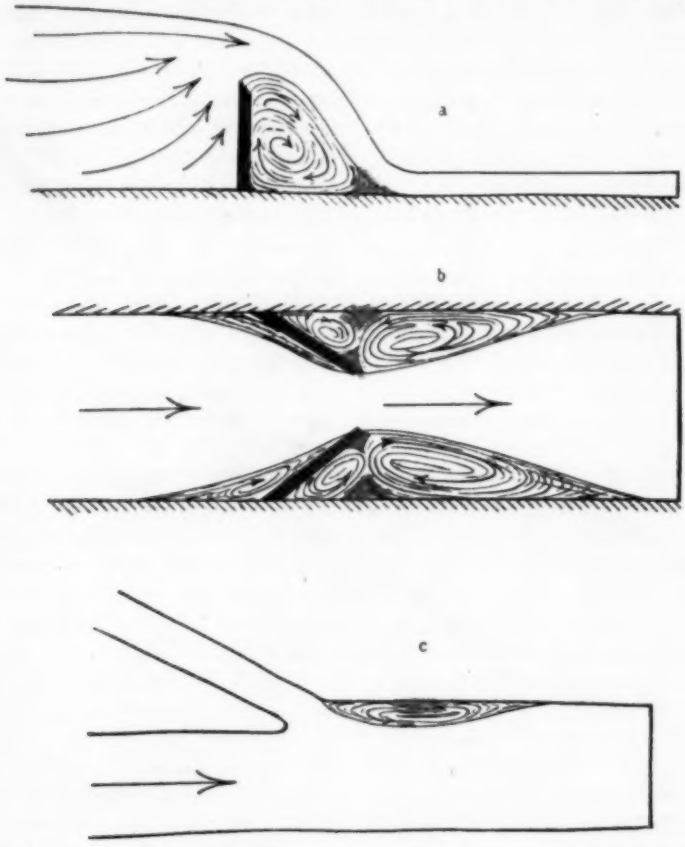
length and like the rest in color, we can expect, it goes without saying, no definite framework of platelets, and in fact it is either completely absent or occurs only partially. This red thrombus resembles in its microscopic structure essentially a post-mortem clot, and consists of an irregularly arranged mass of red and white corpuscles, blood-platelets and fibrin. It must be admitted that here the leukocytes and blood-platelets have a tendency to form themselves into masses, but there is no attempt at the formation of lamellae. The fibrin frequently shows striped thickenings running in definite directions. These may be the results of currents in the plasma, for although the column of blood has been checked it is still connected in manometer-like fashion with the rest of the vascular system. They may also merely be the expression of the movements of the fluids induced by the coagulation.

Thus an essential difference exists between the *Kopfteil* and *Schwanzteil* of the thrombus, which it must be said are connected together by a more or less transitional zone. This difference we know must depend on the fact that the *Kopfteil* is formed in flowing blood, the *Schwanzteil* in blood at a standstill. This brings up the question then of *why a white thrombus is built up from platelets in the flowing stream and a red thrombus when the blood-flow has ceased.*

MECHANICS OF THROMBUS FORMATION

After the corpuscular nature of white thrombi was recognized by Zahn, von Recklinghausen directed attention to the important bearing the slowing of, or rather the formation of eddies in the blood-stream, had on this question. His work has given considerable support to the well-known teaching of Virchow regarding the mechanical genesis of thrombi. When we compare the blood-stream with a river which has countless small particles of different specific gravity swimming about in its waters, we are driven to the conclusion that there must be some optimum velocity which will bring about a local aggregation of particles, such as we have seen by the microscope to occur in the building of thrombi. Neither by a rapidly flowing stream nor by complete stoppage of the current can such a heaping up be brought about. Thus the blood must flow slowly, or, to put it in a better way, must flow differently from the way it did before. It is noteworthy that deposits of sand in a river occur by preference where a widening or a deepening of the river bed exists. It is not a uniform slowing, but the inequality of the local conditions which predisposes to this extraordinary deposit. The insertion of a dam or barrier, by introducing counter currents and eddies, is also an important predisposing cause. I have made an effort to explain this building of sand banks on purely physical lines, and in this I have had the assistance of Prof. Dr. Rehbock in whose laboratory the experiments were

carried out. If a weir is introduced in a stream of fluid, then by the interference with the current so induced an unusually long *"Walze"* (or helix), as it is called technically, is produced in which a portion of the fluid flows backward. And not only that, but fine particles suspended in the water (we used sawdust for the purpose) all deposited in ripple



Diagrams showing (a) eddy formation behind a weir; (b) eddy formation in front of, behind and beneath obliquely-placed weirs; (c) eddy formed at the point of junction of two streams of unequal size. (From Aschoff: Thrombose und Sandbankbildung., Ziegler's Beitr., 1912, lii, 209.)

and net-like elevations in the regions of this *"Walze."* By means of obliquely placed weirs two *"Walzen"* can be produced, the one proximal, the other distal to the obstruction. It is at the point of contact of the two that thrombi arise, and, as Ferge also found, the thrombus is not built close under the valves of the veins. (See Fig. 1.)

Finally the question of sand-bank building by the flowing together of two unequal streams must be considered from the physical standpoint. When we study what occurs in a pipe when this state of affairs is brought about, it will be found that a slowing of the stream in the so-called zone of transition occurs. If the stream from the smaller vessel flows very slowly a sand-bank is formed at its mouth. This agrees very well with what is found in human pathology.

All these experiments are, on account of the number of factors outside our control, merely suggestive. We can take no account of the part played by the living blood and the living vessel wall. We can only prove the gross results and ask what alterations of the blood-stream, in the form of sudden slowing, backward flowing and eddy making, are found in the places where in the human subject autochthonous thrombi have a tendency to grow.

LOCAL CONDITIONS FAVORING THROMBOSIS

The special tendency to slowing of the blood-stream is quite properly insisted on by every one who has written on the subject. It is quite clear that some very special and unusual condition must be present in the arterial system to slow the blood long enough to allow a deposit of blood-platelets to occur. As Virchow pointed out long ago, and as is now generally recognized, there are certain situations in the venous systems which are especially predisposed to thrombus formation. Among these the veins of the leg, the proximal part of the femoral vein where the large valves are present, the pelvic plexus, the venous network of the dura mater, and the auricles may be taken as examples. There are besides four conditions each of which, alone or in combination, has to do with the localization of thrombosis. First, there is continued overpressure on the wall of a vein, such as occurs in the veins of the leg from the pressure of the column of blood when the body is upright, or such as is brought about in the venous plexus of the pelvis by downward pressure of the intestines. This overpressure tends to physiological widening, and, finally, may terminate in a physiological thrombosis. I refer to thrombus formation in varicose veins in the lower limbs and to the thrombosis in the prostatic plexus in old age which is so usual as to be practically physiological. Thrombus building in the vaginal plexus, which may be the origin of a spreading thrombosis or be a nidus of secondary infection, has not had sufficient attention paid to it.

A second condition is the widening which obtains in the auricles, and also occurs in the veins at each valvular sinus. Ferge's recent experiments have confirmed the work of Kölliker and Epstein, who discovered that the musculature of the vein wall can be practically absent in the region of the valvular sinuses, so that backward pressure must

be followed by an ampulla-like widening above the valve. Further, the possibility of a backward pulsation in the veins, the so-called venous pulse, which often is most marked at the proximal end of the femoral vein, must not be lost sight of. Lastly, when the body is lying prone, certain local conditions affect directly certain veins with a known tendency to thrombosis. For example, the femoral vein just where the large valves are present, lies close under Poupart's ligament, and the importance of the bend so brought about must not be underestimated. The iliac vein shortly after the junction of the hypogastric vein, also comes into the same category, and its tendency to thrombosis is also to be referred to the bend in the path in which the blood flows.

Naturally, these local factors play an even greater part when any defect in the heart's action leads to a general slowing in the venous system. In such a case the posture of the patient exerts a further and additional influence. In certain conditions the posture has directly to do with the left or right sided position of the thrombus, the limb which lies lower having a greater tendency to become thrombosed. When lying on the back the increased compression of the left iliac vein by the arterial trunks (right iliac, middle sacral and left hypogastric arteries) has a direct influence in slowing the stream, and explains the well-known frequency of thrombosis in the left lower limb. Another point, too, is of interest. When a double sided thrombosis of the veins of the lower limbs occurs, the following characteristic conditions can often be found. The thrombosis on the right side extends up to Poupart's ligament, whereas on the left side it extends up to the point of compression of the left iliac vein by the right iliac artery.

The deposition of blood-platelets in all the above mentioned examples of thrombosis is easily understood. It is not the stagnation but the retardation which brings about the thrombosis, and must be reckoned as the direct cause of the deposition. Eberth and Schimmelbusch have given to this type the name of conglutination thrombosis. They took this descriptive name from a phenomenon which occurs in the heaped-up mass of platelets, namely, the cementing of the platelets together. This phenomenon can also quite truly be called agglutination, so the term agglutination thrombosis is also a suitable one.

Between accumulation of platelets on the one hand and conglutination or agglutination on the other there exist very special relationships.

We do not know as yet anything of the phenomena which precede agglutination and which are the very earliest factors in thrombosis. We cannot say whether they are of a type long known to workers in bacteriology or serology, or whether they are chemicophysical. We suppose at least that a special degree of viscosity must be present to allow the masses of platelets to form so easily, whereas precipitation phenomena

in the sense of agglutination can also play a rôle. It is also quite conceivable that a mass of platelets, once it is formed, can exert an influence on other platelets flowing past it. The agglutinability of the platelets depends, of course, on the chemicophysical characters of the fluid in which they are, in this case, the blood-plasma.

Thus we see that alteration in the blood-stream is only one condition in the making of a thrombosis, the second condition being dependent on the agglutinability of the platelets themselves. Unfortunately, we have no knowledge of spontaneous changes occurring in this property of agglutinability. This question, chiefly from the experimental side, is considered by Achard and Aynaud.

It is evident that the number of the platelets is an important matter to be considered in relation to thrombosis. Judging from the literature it seems clear that the number of platelets—their exact enumeration is no light matter — undergoes great variations in cachectic states. We know too, that after experimental anemia the number of blood-platelets increases rapidly, coincident, as has been shown by Ogata, with increase in the number of giant cells in the marrow. It is, therefore, quite feasible to suppose that subsequent to loss of blood at operations or during parturition, an increased tendency to thrombosis can be brought about, although it cannot at all be said that an increase in the number of platelets can by itself alone bring on thrombosis.

VASCULAR CHANGES AS INFLUENCING THROMBOSIS

We have up to this point considered two of the conditions which have to do with the building of thrombi, namely, changes in the blood-stream, and qualitative and quantitative alterations in the platelets. We will now take up a third condition, which used to play the chief rôle in the teaching about thrombosis, but whose signification was soon greatly limited by Virchow. I refer to alteration of the vessel wall itself. That this is not the decisive, not to say the only cause of thrombosis of the ordinary type, is made very clear when we can think of an atherosclerotic aorta, which in spite of the most marked changes can remain quite free from thrombi. Here, of course, there is no slowing of the blood-stream. On the other hand, the question arises whether the slowing of the stream can alone, without any accompanying change in the vessel wall, give rise to a thrombosis by deposition. Do the deposited platelets remain bound together only when the endothelium is damaged? Is this damage of the endothelium a third condition which must be fulfilled before thrombosis by deposition can arise, and how must the endothelium be damaged? Covering this point of endothelial damage, the importance of which is always accepted without question, and which is always given great prominence in the literature, we know in reality practically nothing. We

can affirm only one thing, namely, that chronic changes of slight degree in the intimal lining of a vein, as for example fatty changes of the endothelium, can by themselves play no important rôle, since one must so often assume this change to be present without thrombosis resulting. Besides, it is very difficult to understand how a fatty change of the endothelium merely can lead to a deposition of blood-platelets. I can find in the literature no well authenticated cases, in which thrombosis has really been brought about by changes in the endothelium, a factor which is regarded by many authors as of special importance. It is naturally quite another story when rigidity of the wall and valves of the vein, brought about by severe and progressive phlebosclerosis, has interfered with the blood-flow. Changes in the wall of the vein are then not the direct, but only an indirect source of thrombus formation. An analogous state is brought about in the numerous experiments carried out to induce thrombosis by means of corrosives, cutting and the introduction of threads or other foreign elements. These experiments were carried out at an early date by Zahn, Eberth and Schimmelbusch, Welch, and more recently especially by Zurhellen in the institute in Freiburg. Here the rough and mechanical disturbances of the blood-stream resulting from the laceration of the vessel wall, do not play the entire rôle, but the plasmatic exchanges between the fluids of the blood and the lining of the vessel must also be important. When instead of slow retrogressive changes, the intima is suddenly stripped of its endothelial lining, then a reaction inevitably ensues, as occurs in all living tissues, accompanied by a pathological flow of lymph. Then it is, indeed, the alterations in the blood-stream in the region of the injured area which bring about a thrombosis by deposition. It is not made clear by the previous experiments how and whether a direct influence on the blood-platelets as they flow by can possibly be exerted by agencies of an agglutinative or coagulative nature produced by the tissues (L. Loeb, Achard, Aynaud). Nor is it explained whether the connective tissues or the muscle laid bare by the damage at a surface capable of absorption (Morawitz) can bring about the adhesion of the platelets. At any rate, all these artificially produced thrombi present the characteristic structure of thrombi by deposition of the blood-platelets. We must allow that up to the present we have not been well enough informed concerning the first and absolute beginnings of thrombus formation in the case of autochthonous thrombi in the human subject. Unfortunately in this connection we can hardly ever obtain fresh enough material, since, of course, we must eliminate all post-mortem coagulation, which naturally can occur in the thrombus itself.

We must endeavor to get a clear idea of why the adhesion of the platelets to the vessel wall occurs in autochthonous thrombosis, since we

assume that this deposition of platelets is the first procedure. It may be quite possible that the endothelium dies as a consequence of being covered over by the platelets. Fibrin is in this way set free and cements the masses of platelets to the wall. Or the platelets after lying on the wall for some time may themselves die, and then coagulation on and between the endothelial cells can ensue. At any rate, it is necessary that an interaction of a certain duration occur between the platelets and the endothelium in order that the material may remain stationary and, so to say, be fixed there. If slowing of the blood-stream and alteration in the conditions of the platelets are to figure as the direct facts in thrombus formation, then we must consider as indirect factors changes in the wall, alterations in the cardiac action and loss of blood during operations.

In considering the vital question, as to why a white thrombus occurs in the circulating blood, and a red thrombus only when the stream is stationary, I have already attempted to answer the first part and shall now proceed to the second. I have already mentioned that a red thrombus arises only when the lumen of the vessel is sufficiently closed by a white thrombus to bring the blood-stream to a standstill in the peripheral portion of the vein. This stationary column of blood coagulates. But under the microscope this red thrombus, dense in the neighborhood of the *Kopfteil*, while peripherally becoming more and more spongy, and, finally, almost fluid in consistence, can scarcely be differentiated from postmortem clotting. The microscope shows, however, that the denser portion is richer in fibrin and leukocytes, and suggestions of lamellae can here and there be recognized. As we travel more to the periphery the structure of the thrombotic mass more and more approaches that of the normal blood. Microscopically, it is also true that the boundary between the white and the red portions is as hard to recognize as that between the slowing and final complete stoppage of the blood-stream. From these points we can also explain why this column of stagnating blood does go on to coagulation, in contradiction to the well-known findings of Baumgarten, who observed that the column of blood contained in a doubly ligated portion of a vessel did not coagulate. We must take into consideration that, as has been already mentioned, coagulation phenomena can also be observed to have taken place in the blood found occupying the gaps in the white portion of a thrombus. These phenomena very probably must be referred back, judging from the characteristic situation of the fibrin network, to an interaction between the fibrin ferment set free by the death of the platelets and the stationary blood-plasma round about them.

Of course, the amount of fibrin ferment set free in the white *Kopfteil* will be very considerable. It can advance by diffusion into the caudal red portion of the stagnating blood-column, which is richer in leukocytes

and platelets and develop its action. We know, as Baumgarten has stated, that the cause of the blood remaining fluid in a doubly ligated segment is that the blood dies relatively slowly. Conversely, we can presuppose a very rapid death to occur in a structure rich in ferment, as the blood-platelets are, and so the clotting of a column of blood in stagnation becomes intelligible. I will not consider here the processes which precede clotting, and above all I cannot discuss the question of where the forces which bring about clotting have their origin. Nor can I stop to inquire whether the source of thrombokinase is to be sought for only in the thrombocytes and leukocytes, or in the erythrocytes as well. I would prefer to refer you to the well known and very clearly expressed papers of Morawitz.[3]

I pass over here the finer morphology of the processes preceding clotting, and would only mention that the "centers of coagulation," so-called, are to be regarded in my opinion as acting both chemically and physically. In the origin of the red thrombus we are also dealing not with the separation of the formed elements of the blood, which practically does not occur, but more especially with crystallization of fibrin as in ordinary clotting. Thus we have a coagulation of the blood as a whole, and not merely the deposition of some parts of it. This view has already been suggested by previous authors, who have put forward the name coagulation thrombosis, in contra-distinction to conglutination and agglutination thrombosis.

Just as in deposition thrombosis the slowing of the blood-stream and the relations of the platelets as to number and viscosity are the essentials, so in clotting *"en masse"* the stoppage of the stream and the increase of fibrin ferment are the important factors. It cannot be denied that an intimate relationship exists between the heaping up of platelets, the agglutination and the clotting, i. e., the coagulation. He must conclude that agglutination is, so to speak, only a means of creating in the circulating blood conditions which are necessary to allow of the crystallizing out of fibrin, a process which cannot occur in the circulating blood under ordinary conditions.

But it is quite evident that these two phenomena (agglutination and crystallizing out of fibrin) do not mean the same thing and must be kept genetically apart, since each can occur without the other. In the case of ordinary autochthonous thrombosis, with which we have been dealing up till now, the deposition and coagulation processes are closely related. The process of deposition brings about the coagulation, and is, so to speak, the indirect source of it. Then the further question arises as to whether coagulation thrombosis can occur apart from the process of deposition, and what may be the indirect causes influencing them both.

---

3. Morawitz: Blutgerinnung, Handb. d. Biochemie d. Menschen u. d. Tiere, 1908.

It is important in this connection to remember that while thrombosis by deposition can be brought about readily at any time, a primary coagulation thrombosis is very difficult to induce. The stoppage of the blood-stream alone does not suffice, as Baumgarten's experiments have shown, for the ferment does not develop quickly enough. Also optimal relations must be established between the rate of stream and amount of ferment present, to initiate the process of clotting. Even the flowing blood, however, can be made to clot, if a large quantity of ferment is introduced all at once, either by the injection of pure fibrin ferment or by artificial transfusion. Of course, control by the microscope is essential in such experiments to ascertain whether the solidification of the blood depends really on clotting of fibrin, and is not merely a precipitation, such as occurs when a substance which coagulates albumin is introduced.

To the question of these precipitation thromboses, and other related processes, I will return later.

Two other processes must be mentioned here which undoubtedly have relationships with coagulation thrombosis. I refer to ligature and infection. Since, as we shall see, both of these can also induce thrombosis by deposition, we can now inquire how ligature and infection, respectively, act, and what is their significance from the point of view of thrombosis.

The facts in the case of ligature are comparatively simple. If done carefully so that considerable damage to the intima is avoided, and the portion included by the ligature is so small as not to interfere with the movement of the blood, no clotting need necessarily occur, a fact which we have noticed elsewhere in connection with transplantation of blood-vessels. At other times we find thrombus formation, often quite scantily, however, in the region of the intimal damage, where the fibrin ferment arising from the injured wall can act on the stationary blood in its neighborhood. Or at the margin of the ligatured part of the vessel a true deposition thrombosis may arise as a result of local eddy formation. In this way the area may finally be separated off completely from the blood-stream, and the column of blood thus shut off passes into a condition of coagulation thrombosis, like the *Schwanzteil* of an autochthonous thrombus.

RELATION OF INFECTION TO THROMBOSIS

Much more difficult to explain is the question of infection to thrombosis, nowadays considered so important. It is especially from the findings of clinicians that the idea has been put forward that not only most, but as some will have it, all forms of thrombus formation are to be referred back to infection. It is unfortunate for the advancement of pathological anatomy that when as a sequel of thrombosis cases come to autopsy, an infection often does not exist or at any rate can no longer be

recognized. Concerning infective thrombosis, I would say only that from my own researches I am of opinion that what occurs generally is a secondary infection of an already existing simple thrombus; for example, in the site of an operation or in the area of uterine wall laid bare by the removal of the placenta. Of course, it is not impossible that a primary inflammatory process in the surrounding tissues can implicate and extend through the wall of a vein and a thrombosis occur from the changes in the blood-stream arising in that way. Of more importance, however, than these locally infective thrombi are those of septic character which arise at a distance from the site of an operation; for example, septic thrombosis of the femoral vein following operative interference with the pelvic connective tissue. Here our observations have shown that a typical thrombosis by deposition always occurs, the organisms themselves being found enclosed here and there in the framework of the thrombus. One must necessarily conclude from this histological picture that the organisms circulating in the blood are only, so to speak, secondarily enclosed in the thrombus while it is being built. The thrombus is otherwise identical in structure with a simple one. We must, therefore, assume that here the thrombosis has a similar origin as in the non-infective type, namely, a slowing of the blood-stream, and that the organisms themselves are not the causative agents. The latter are merely accidentally enclosed, but are dangerous, since by their multiplication they convert a simple thrombus into a septic one, and may bring on all the consequences of septic emboli. The following abridged account may be given of the complicated picture which is produced by the infection occurring in the thrombus in a vein.

1. A local infective thrombosis in the region of an operation or in the neighborhood of the placenta can arise: (a) Through infection of thrombi already present, to which an inflammation of the wall has arisen secondarily (thrombophlebitis). (b) By an infection progressing along the vessel wall, which induces first of all a primary phlebitis and is followed by a secondary thrombosis by deposition (phlebitic thrombosis). (c) By means of slowing of the blood-stream and thrombus formation in the commencements of the veins followed by thrombosis by deposition in the larger veins, induced by toxic or inflammatory causes, which subsequently become infected.

2. An infective embolic thrombosis arising at a distance is in most cases the sequel of an ordinary primary thrombosis, which acquires its infective character secondarily by multiplication of micro-organisms in the blood shut in the interior of the thrombus. Experimentally it would appear probable that only exceptionally the organisms separate themselves from the blood, directly infect the wall and so bring about a secondary thrombosis.

3. All septic thrombi can extend on account of the inflammatory changes in the wall. These are not confined to the area in contact with the clot, owing to the toxins being diffusible.

Since in the case of distant thrombosis the infection usually arises in already existing thrombi, and cannot be regarded as the source of the thrombosis, but rather as an accompanying or induced phenomenon, we

must inquire why, especially after operative procedures or in already established infection, thrombosis at a distance is of frequent occurrence. It is well known that in acutely progressing septic cases thrombosis at a distance is much rarer than in subacute or chronic cases. That in itself speaks against a direct action of infection.

Thrombosis of the femoral vein, among the thromboses at a distance, interests us most, since it is the special origin of fatal lung emboli. Here extremely varying factors work together — loss of blood at operation, weakening of the heart, general prostration, confinement to bed, the influencing which every pelvic operation has on the return of the venous blood from the lower limbs to the heart, because the venous plexus of the pelvis acts as a manometer to this great area of blood.

Finally, the possible changes in blood platelets themselves induced by the infection must also be considered. In reality we have no positive knowledge on the question whether the bacterial toxins can increase the viscosity of the blood-platelets.

I have taken up so much time with the static or autochthonous thrombosis, because it seems to be the most important variety, and is, as a rule, easily recognized both by the clinician and the pathologist. With its description, however, the question of thrombosis is in no way exhausted. I have already made special mention of the work of my American colleagues, Flexner and Loeb, who, by using toxins, studied the processes in the living body which accompany the toxic thrombosis described previously by Naunyn, Landois, Ponfick, Silbermann, Kaufmann, Falkenberg and others, as resulting from blood transfusion and specific blood-poisons, and by Takowski, Lubarsch, Bardeleben, etc., as a consequence of injection of bacteria and bacterial toxins. Recently in Germany, Dietrich and my own pupil, Kusama, have carried out such experiments, and I would like to make a few remarks about Kusama's work which has only lately been completed.

CAPILLARY THROMBOSIS

Since the independent elements of the blood are influenced in the highest degree in all these thromboses of toxic or infective origin, while the conditions in the blood-stream itself remain essentially the same, I have called this form of thrombosis "thrombosis by alteration of the blood," in contradistinction to "static thrombosis." Further, as you will hear immediately, the initial thrombus building occurs in the capillary system, and so one can also call this form capillary thrombosis, in contradistinction to that occurring in the larger vessels.

In continuation of the work of Flexner and Loeb, who, by injecting heterologous serums, have explained the significance of the agglutination of erythrocytes, the deposition of platelets and the formation of fibrin

in thrombosis, and in connection also with the work of Dietrich, who, above all, has tried to elucidate the mode of action of the end-products of hemolysis, namely, the blood-cells on one side, and the agglutinins and precipitins on the other, my pupils and I have tried to recognize the factors which are essential for the production of thrombi in the circulating blood.

So far, our experiments agree more with those of Flexner and Loeb than with those of Dietrich, who studied chiefly the effects on the stationary blood-column. We began by investigating the effects of homologous serums on rabbits. I shall take no notice here of the clinical symptoms induced nor of the altered coagulability of the blood, but would chiefly confine myself to the morphological findings. Rabbits showing no signs of illness, were killed twenty minutes after the injection of a homologous serum. Examinations of the organs just after death showed in the places predisposed to capillary thrombosis — for example, the capillaries and post-capillary venules of the lungs — very abundant thrombus formation. The thrombi consisted in parts exclusively of blood-platelets, packed together but well preserved, in other parts of a mixture of platelets and red-cell stromata. When the fixation was good, fibrin was absolutely absent or only to be found in traces inside the thrombi. On what, then, do these thrombi, formed from platelets and red-cell stromata, depend? They might be a direct effect of the serum injected, which both agglutinated the platelets, and after hemolyzing the erythrocytes, agglutinated their stromata. To answer the question, Kusama hemolyzed red corpuscles by washing them thoroughly with distilled water and then injected the solution of hemoglobin. When the animals were killed, ten to twenty minutes after such an injection, thrombi composed of blood-platelets could be found in the vessels of the lungs. On the other hand no thrombi composed of stromata were met with. A strongly agglutinative effect on the blood-platelets is thus exercised by hemoglobin circulating free in the plasma. It is noteworthy that in these experiments the platelets also remain very well preserved. By simple washing of the stromata with distilled water a distinct effect on them is produced. If a suspension of these were injected into rabbits and the animals killed after a definite time, thrombi composed of stromata were not only found to have arisen by the agglutination of these cell remnants, but also undoubted thrombi from blood-platelets were found. The increased agglutinability of the platelets must have been brought about by living substances produced by the solution of stromata in the blood plasma. If a suspension of stromata, which had been heated at 56 to 58 C. for forty minutes were injected, no such blood-platelet thrombosis ensues. So it seems that the living substances set free by the dissolving of the stromata in the plasma, are of great

importance in effecting an increased agglutinability of the platelets. Experiments in animals which were allowed to live a longer period show that the stromata do dissolve in the circulating blood. In such experiments thrombi composed of stromata were found to have completely disappeared, so they must either have dissolved, or as is much less likely, have been washed away by the blood-stream. Thrombi composed of blood-platelets are also not seen in these animals, and from the fact that the individual plaques were still well preserved, it seems reasonable to suppose that they again enter the circulation.

Now let us consider how a foreign or heterologous serum acts. In Loeb's work, dog's serum was taken as the representative of strongly hemolytic and coagulating serums, while ox serum represented the group of strongly agglutinative serums. After the injection of dog's serum the rabbits died within a few minutes, showing characteristic symptoms (excitements, coma, convulsions, projecting eyeballs, abdominal distention, etc.). At the autopsy, fresh clotting was found in the lung arteries, in the right side of the heart, in the vena cava and in the lung veins, and death appeared certainly the result of these pathological findings. When, however, the experiment was carried out, after carefully opening the pericardium, it was seen that the clinical symptoms and the stoppage of breathing occurred while the heart continued to beat and the blood remained quite fluid. Microscopically, just as in the case of injection of a homologous serum, thrombi composed of stromata and platelets are to be seen in the lung capillaries, in the post-capillary venules, and also in the precapillary arterioles. But these thrombi differ from those discussed before in that the platelets show definite disintegration. Thus, not only agglutination, but destructive processes have been produced. This latter occurrence can only be caused by the heterologous serum itself, and the marked increase in the coagulability of the blood must also be referred to the disintegration of the platelets. One might be inclined to think that death might be produced by the blood-platelet thrombi, whereas the coagulation of the blood alone could not be the cause. But in this connection it is easy to show that where an animal has been given a non-lethal dose of dog's serum and been killed many hours later, capillary thromboses are just as marked as when the animal is killed at a much earlier stage. So, then, the fatal result can be referred neither to the blood coagulation nor to the capillary thrombosis. It must depend on some other changes in the blood, or increased viscosity of the plasma, or direct toxic action on the central nervous system.

The fact proved by Loeb's experiments with leech extract (hirudin) that the clotting is not responsible for death, was also confirmed by Kusama. Many rabbits, after injection of hirudin, died, although the blood postmortem was found quite fluid. The blood platelets seem to be

diminished, since, according to all accounts, hirudin has a hindering action on the hemolytic and agglutinative properties of a heterologous serum. Experiments with ox serum give quite similar results. Kusama was unable to confirm the difference on which Loeb laid emphasis, namely, that by injection of ox serum marked clotting is absent. But he fully confirmed another point on which Loeb laid stress, the fact that agglutination plays a special rôle in all these experiments. In contrast to what occurs with dog's serum, many thrombi formed from stromata are found, but few composed of blood platelets. Also the platelets are not altered in the same way as after injection of dog's serum. It should be explained in addition that in some of the experiments with ox serum the coagulability of the blood did not show nearly so marked an increase as after injection of dog's serum.

Since as a matter of fact blood-platelet thrombosis, which in most researches has been given only a subsidiary place, is a result of hemolysis, we should be able to reproduce a similar thrombosis by injecting other hemolytic agents.

With this end in view, researches with ether were undertaken. It was previously believed that injection of ether was followed by a direct clotting of the blood, but as the result of experiments carried out by myself I have been opposed to this view for a long time. Then Loeb showed that injection of ether brought about an agglutination and destruction of erythrocytes, and considered the agglutination the cause of the death of the red cells. Our own experiments confirm this in so far that ether is hemolytic just like dog's serum, and brings about thrombosis by deposition of stromata and platelets. But no peculiar or special red-cell thrombosis is called out by its injection. Ether is in addition directly destructive to the blood-platelets, and so the increased coagulability of the blood is explained. Glycerin and distilled water act in a manner similar to ether.

On the contrary, other blood-poisons, ricin for example, act in a surprising and entirely different way. Kusama's experiments showed that practically no hemolysis of erythrocytes occurred. Hence no noticeable thrombosis from blood-platelets or stromata ensued.

What does occur is really this: Ricin acts destructively on the myeloid and lymphoid cells of the spleen, bone-marrow and liver, and on the endothelial lining of the vessels. The fragments of cells so produced pack together in the capillaries of the spleen, bone-marrow and liver, and induce thrombosis. A definite increase in the coagulability of the blood does not occur as a rule. The coagulation of fibrin is secondary, just as in the case of thrombosis from stromata and blood-platelets.

Finally, experiments with bacterial cultures seem to be especially important. By injecting dead cultures of the *Bacillus typhosus* a definite

fibrin thrombosis was produced, especially in the lung capillaries, but also in the capillaries of many other organs. In these thrombi leukocytes were enclosed, either broken down or well preserved. Destruction of myeloid and lymphatic cells was seen in the hemopoietic system, but not nearly to such a degree as after ricin. It was very strikingly brought out that these small thrombi from cell fragments (Trümmerthrombi) were accompanied by such a considerable deposit of fibrin. It seemed as if to explain it the bacteria must also be considered as foreign bodies. It is very difficult to extract the endotoxin of the *Bacillus typhosus*, so the experiment could only be repeated with typhoid bacilli heated for a much longer time. The results were similar, but not nearly so marked as when bacilli only killed by heat were injected. Also a previous immunizing of the animal against *Bacillus typhosus* had no obvious effect in decreasing the amount of thrombus building and of fibrin deposition. Dead cultures of the bacillus of dysentery and of staphylococci behave in exactly analogous fashion. In the case of cultures of the latter organism the destructive action on the cells of hemopoietic organs is very slight, whereas the formation of thrombi from leukocytes and fibrin in the capillaries of the lungs and other organs is very marked. The leukocytes always contained very numerous cocci.

In conclusion, I would say that the effect of bacterial injection is merely the production of leukocytic thrombi, and deposition of fibrin follows closely on this process. The definite occurrence of leukocytic thrombi, although they are only temporary, explains the marked leukopenia which can be recognized one to four hours after the injection, in the peripheral blood, in most of the experiments.

To decide what part of the process could be referred to the mere presence of foreign elements in the blood, other animals were injected with a solution of India ink. The result was a definite thrombosis from blood-platelets in the lung capillaries. Many leukocytes were enclosed in the thrombus, but no *deposit of fibrin* occurred. This result is different from that produced by bacteria, in that deposition of fibrin was absent, a blood-platelet thrombosis being the chief result. No marked destruction of leukocytes was seen. Phagocytosis of particles of India ink, although it was present, was not so marked as in the case of cocci. The packing of the leukocytes inside the lung capillaries produced a leukopenia just as after injection of dead bacterial cultures.

Collargol was also injected and produced a thrombosis chiefly from blood-platelets, agglutination of leukocytes being practically absent. Olive oil gave a similar picture. Injection of ground rice was followed by the same result induced by bacteria, in that, whereas blood-platelet thrombosis did not occur, thrombi from leukocytes and fibrin were to be found in the lung capillaries.

Thus all foreign elements do not act in the same way. Substances like India ink, collargol and olive oil must have some attraction for platelets, whereas the ground rice and dead bacteria have none. The first three produce thrombosis from blood-platelets, the latter groups only leukocytic thrombosis and the greater the destruction of leukocytes and platelets the sooner is fibrin deposited.

CONCLUSIONS

What result, then, can we glean from all these experiments? We have seen that different poisons, whether hemolytic in action or not, bring about distinctive forms of capillary thrombosis, among which we can differentiate the following types:

I. Blood-Platelet Thrombosis.—This arises by intravenous injection of a homologous serum, of solutions of hemoglobin and stromata from homologous blood, of heterologous serums, ether, glycerin, distilled water, India ink, collargol and olive oil. The thrombi arise in the capillaries of lungs, liver and spleen.

II. Thrombosis from Blood-Stromata.—This occurs in pure form only, when very marked hemolysis occurs in the blood-stream as a result of the action of hemolytic agents. Otherwise it occurs only in conjunction with blood-platelet thrombosis.

III. Thrombosis from Fragments of the Blood Elements (Bluttrümmerthrombose).—Disintegration of the elements of the blood occurs by introduction of ricin, bacterial toxins, etc., which destroy the leukocytes, lymphocytes, macrophages of the spleen, liver and bone-marrow.

The fragments, in conjunction with scanty fragments of erythrocytes, roll themselves together to form thrombi in the spleen and liver. Fibrin is then subsequently deposited there.

IV. Leukocytic Thrombosis.—The polymorphic and mononuclear leukocytes phagocyte the foreign elements introduced into the blood-stream, and are stopped and held back in the capillaries of the internal organs, especially the lungs, spleen and liver, so that a leukopenia results in the peripheral circulation. This process is especially brought out by bacteria, but also by various indifferent foreign substances like particles of ink and starch.

V. Fibrin Thrombosis.—Although by injecting stromata or foreign serums the coagulability of the blood can be increased, no deposit of fibrin occurs. Deposit of fibrin occurs only secondarily, as a sequel of the types of thrombosis already described.

Let us take, then, a final glance over the subject of static and toxic thrombosis. We see that neither endothelial damage, on which so much stress was previously laid, nor a direct coagulation of the blood, play any rôle. Only two real reasons for the thrombosis require to be con-

sidered, and their merits weighed one against the other. I refer to the changes in the blood elements themselves, and to the slowing of the blood-stream. Neither of these two factors can be excluded, as even in the experiments where thrombi of different sorts are produced by heightening the agglutinability of the blood-platelets by the disintegration of white or red corpuscles, these thrombi can occur only where *normally* a marked slowing of the blood-stream is present; for example, in the capillaries of the lungs, liver and spleen. But even by very marked destruction of the blood elements it is impossible to produce thrombi by deposition in the larger veins when no slowing of the current occurs.

An additional factor, therefore, needs to be considered, especially in cases in which the thrombi are localized in the large veins, and where the above-mentioned capillary regions of the lungs, liver and spleen generally implicated in toxic thrombosis remain free. In such a case the increased agglutinability of the blood-platelets cannot be the direct exciting cause, but a definite and recognizable slowing of the blood-stream must first of all occur.

So we see from all these experiments that the slowing of the blood-stream and the alteration of the blood elements themselves, especially alterations of the platelets, are the chief factors in the production, not only of the static, but of toxic thrombosis, so far as we are concerned with thrombus formation in the flowing blood-stream. In static and similar types of thrombi the slowing of the blood-stream is of prime importance, while for the toxic varieties the changes in the blood elements have the dominating influence.

Nevertheless, we must ask ourselves whether very important and intimate relationship do not exist between thrombosis and coagulation of fibrin. There is no doubt that, with a few exceptions, coagulation of fibrin as a rule sooner or later inevitably follows thromboses. So the division of the process of thrombosis, as set forth by Loeb for the lower animals, is now quite clear. The first stage is the erection of a morphological structure by a process of *agglutination*. Fibrin ferment is obtained from the agglutinated elements and cements them together by coagulation. This is the second phase.

Therefore, in the process of thrombosis in human beings the phylogenetic development of the complicated mechanism of coagulation is repeated.

STUDIES OF BLOOD-PRESSURE IN STATES OF EXCITEMENT AND DEPRESSION *

M. C. HAWLEY, M.D.
WATERTOWN, ILL.

This paper treats of blood-pressure, work done recently under the direction of Dr. H. D. Singer, at the State Psychopathic Institute, in cases of mania, depression and agitation. The sphygmomanometer used was that of Erlanger. The number of cases investigated is not large, neither do the extremely-marked examples of mania or agitation figure among those cited because it is impossible to get a reading in such cases. The reason is that as one is dealing with a delicate and sensitive machine which registers blood-pressure graphically, the procedure would probably result in disaster and the reading be worthless. When the typical is sifted out and the impossible is eliminated, surprisingly few cases are left to be dealt with. These few cases, then, were taken without regard to the age of the patient or to the condition of the vascular system; the readings were all taken at regular intervals and at the same time of day, and the patients were all in the same position, namely, lying on the operating-table. In fact, as far as possible, all the conditions were made constant in each one of the cases. Several readings from the same patient were taken, and this I deem quite important, as we find that the blood-pressure at different times and in some patients varies quite considerably. No accurate deductions could be made from one reading. One might quite erroneously consider a systolic pressure of 200 mm., such as was seen in one of the cases of depression, as a complete confirmation of the accepted view that in cases of depression high readings are obtained and, therefore, take no other reading, when as a matter of fact a day or so later the systolic pressure in this same patient was 140 mm. Again, having the graphic representation of the blood-pressure, one can quite accurately ascertain the diastolic as well as the systolic pressure, and thus find the pulse-pressure quite an important thing to have recorded. The graphic method also records the amplitude of the wave. The observation of this has led to certain views concerning the tracings which will be taken up later. Some marked variations are noticeable.

Having obliterated the pulse by the inflation of the cuff, the air was allowed slowly to escape until the needle began to oscillate when the

* From the Illinois State Psychopathic Institute; read before the Illinois State Hospitals Medical Association, Elgin, Ill., April 24, 1913.

* Submitted for publication June 30, 1913.

height of the mercury in the tube was taken. The finger also was used to catch the returning pulse, and in each instance the pulse was caught after the other observation was made. The difference in time between these two observations decreases as the blood-pressure rises. Hence, it might be said that the readings quoted are slightly higher than they would have been had we used other types of sphygmomanometer. The time is given in seconds measured by an electric metronome.

In reviewing the literature I find in Ziehen's "Lehrbuch" several references which will be considered. Ziehen accepts what Dr. Craig[1] has to say. The latter investigator conducted several experiments to find out first, whether or not certain forms of insanity fall under different heads as far as blood-pressure is concerned, and second, if they can be arranged in different groups, whether the altered blood-pressure is a cause or a result. He discovered that in a general way insanities fall into two groups, namely, that persons under excitement have a low pressure and those under depression have a high pressure. His investigations were all made at the same time of day; no drugs were used; the condition of the bowels was noted, and the cases of known vascular and kidney disturbance were eliminated. For check, he used normal persons with blood-pressures of 120 to 125 mm., and even here he found variations. For instance, those of excitable temperament gave lower and those of apathetic temperament higher pressures. In twenty-one women with acute melancholia he found a pressure of 150 mm. In fifteen men with acute melancholia he found a pressure of 140 to 145 mm. In twenty-two excited women patients he found a pressure of 105 to 110 mm. In eleven excited men patients, pressures of 105 to 110 mm., and some as low as 95 and 100 mm., were found. He cites one case of depression with a blood-pressure of 150, which, on recovery, dropped to 125; this was followed by an excited period with a pressure of 105; recovery ensued and the pressure rose to 125 mm.

In agitation with extreme motor restlessness the pressure is, as a rule, low. In stupor it is high, but in the depression which follows acute mania, which he interprets as an exhaustive condition, he found on the contrary, low pressure. In paretics the pressure is raised during depression and lowered during excitement. In fact, it seems that in all cases of affective disturbance there are oscillations of pressure, whereas in cases of paranoia, where delusions are only ideational, there are no variations from normal. He recites cases of aortic regurgitation (low pressure) in which the patients, on becoming insane, usually presented manic states, while conversely in cases of mitral regurgitation (high pressure) the insanity takes the form of melancholy.

1. Craig: Lancet, London, June 25, 1898.

While no definite conclusions are made as to a solution of the problem, he considers blood-pressure *per se* is not the cause of insanity; but suggests that insanity and blood-pressure may have a cause in common and cites a case in which the pressure fell *pari passu* as the excitement rose. Conversely, there is no definite proof that insanity causes a change in blood-pressure, and, as an example, he cites paranoia. He considers autointoxication in its causative relations and cites the feelings of depression induced by constipation, and the relief not only of depression but also of blood-pressure, particularly in melancholia, when the constipation is relieved. This is on the theory that the splanchnic system regulates blood-pressure in the brain.

Pilcz[2] quotes Gärtner, whose sphygmomanometer he used, as saying that emotional excitement raises blood-pressure and that the first reading is usually the highest because of the stimulation which results from the procedure — a statement which he can confirm — and adds that certain definite mental diseases were marked exceptions to the rule. In each case he took two readings to allow for psychic influence, except in paretics, who were so demented that they were psychically uninfluenced. Normal pressure Pilcz estimates at 105 to 130 mm. Two hundred and forty cases were taken under observation, and nine hundred to nine hundred and fifty readings were made. Assistants, attendants and paranoiacs were used as controls. Paretics gave normal readings at first, but as dementia progressed the pressure fell as low as 50 in bed-ridden patients. Patients in the depression of paresis gave higher readings. By the pressure-fall in the terminal stages of paresis one is able to prognosticate death, even though at the beginning decided arteriosclerosis were present with very high pressure. In depressed patients he found high pressure, but in simple cases the readings were still within normal limits, though relatively high. This was also true of cases of agitation when under the influence of morphin and in the interval between paroxysms. In circular insanity, patients when depressed gave high readings, and when excited low readings. When a reverse reading was obtained it would seem to prognosticate an on-coming variation in mood. He calls attention to Craig's statement, which he does not understand, that in extreme cases of anxiety the blood-pressure goes down, but agrees with him that the exhaustive stupor following mania gives a low pressure. In stupor, following melancholia, the author found high pressure, but no change in catatonic stupor.

Kramer[3] says one must guard against muscular resistance, for this causes increased blood-pressure. He compares his cases with those of normal persons and finds among the latter, variations in blood-pressure

2. Pilcz: Wein. klin. Wchnschr., 1900, xii.

3. Kramer: München. med. Wchnschr., 1892, No. 6.

between 90 and 180, but thinks these normal persons are not practicable subjects for comparison, as they are subject to various conditions which influence the pressure. He also insists that to arrive at a correct result one must take readings which cover a number of years. He calls attention to the fact that, in many instances, recovering patients take on flesh, and as this bodily change influences blood-pressure, a comparison of the readings taken before and after recovery in such a patient, or comparison of the readings in one case in which symptoms are present with readings in another case taken after recovery, are inaccurate. Although muscular action influences pressure in both anxious states and depressed phases of manic depressive insanity, yet aside from this factor there is a high blood-pressure because of the condition of mental depression, and when this mood changes there follows a change in blood-pressure.

I have made several tracings of blood-pressure in manic, agitated and depressed states, but will give only selected ones from each group. Of the manic phase of manic-depressive insanity, there are ten cases. The number of observations taken in this group varies from one to five in each case, and in instances of more than one reading, observations were taken a week apart. These cases are in part typical, the patients showing psychomotor restlessness and press of speech; other patients were merely stimulable, non-productive and irascible, while others were recovering or recovered. Of the depressed phase of manic-depressive insanity, there are six cases. In some of these cases six readings were taken. One of the patients grew mildly agitated during the taking of the reading and a few were stuporous. In the involutional group there are a number of patients — all over 50 years of age; one 73 years of age. A number of readings here were made in each case. Some of the patients have apparently lost affect, in that they pace the floor and wring the hands less, and they remain clear in mind although delusional and apprehensive. One patient is rather anxious and has motor agitation. While I realize that the cases are too few to warrant making any positive deductions from them, I find so many exceptions to the accepted dictum that I cannot entirely, if at all, agree with it.

In looking over the tracings taken in the manic cases, I was impressed with certain particular features which follow:

1. These tracings without exception show a curve of great amplitude (Fig. 1), more marked in the cases of psychomotor restlessness, but sufficiently demonstrable even in the non-productive cases to distinguish them from tracings obtained in cases from the other groups.

2. After the needle has begun to oscillate it rapidly jumps into full amplitude and the systolic blood-pressure is at once reached. This too is more marked the more evident the restlessness. In the other groups this period of oscillation before reaching systolic pressure is long drawn out.

3. After having begun to oscillate the needle works rapidly to the finish, indicating a rapid pulse.

4. The blood-pressure is raised and the pulse-pressure likewise is high.

Some of the cases selected in this group have arteriosclerosis; some have no sclerosis, at least demonstrable. I find in the former the same sort of curve as in the latter, though the sclerosis is a factor in raising the blood and pulse-pressure still higher in such cases of mania. I arranged in groups the patients in whom arteriosclerosis was and was not present and found the average blood-pressure and pulse-pressure higher in cases of mania in which there was arteriosclerosis than in cases of mania in which there was no arteriosclerosis. One sees in manic cases heightened color, sparkling eyes and a motor adjustment to a general feeling of exhilaration, and it is quite probable that in these tracings there is evidence of altered vasomotor tonus.

Fig. 1.—Pulse-tracing, Case 2. Note large amplitude, early onset and rapid action characteristic of tracings in manic states.

MANIC STATES

I shall present three cases which have both manic symptoms and arteriosclerosis.

REPORT OF CASES

CASE 1.—H., aged 63, has a history of previous attacks; is an exhibitionist; is sleepless, restless and has arteriosclerosis. The systolic pressure is 190 mm., the diastolic pressure 124 and the pulse-pressure 66. In a tracing taken in this case what has been observed as the special feature of the tracing in manic states is shown, namely, high amplitude, early onset of the full oscillation and rapid action. When the length of time that elapses before systolic pressure is reached is compared to the length of time that elapses before the same pressure occurs in depressed cases, there is found in the former only a few seconds, while in the latter as many as thirty seconds elapse.

CASE 2.—G., aged 43, has an attack of a few months' duration; has had several attacks; is irritable, vicious and has slight arteriosclerosis. The systolic pressure is 170, diastolic pressure 98, pulse-pressure 72 in one reading and again systolic pressure 144, diastolic pressure 92 and pulse-pressure 52 in another. The features again observed are large amplitude, early onset and rapid action (Fig. 1). Notice that the pressure varies. We cannot determine whether this

difference is due to the variance in the arteriosclerotic factor or to the manic factor. It was with great difficulty I could catch the systolic pressure because of its early onset after the beginning of oscillation.

CASE 3.—S., aged 43, has had a previous attack; is expansive, talkative and alert. The mania is of a religious type. The patient has arteriosclerosis. Five readings show:

Systolic pressure	Diastolic pressure
150	104
150	100
160	100
148	90
160	100

I had difficulty in catching the systolic pressure, so rapid was the onset of full amplitude.

These tracings do not vary from the others previously cited. The next four cases of mania have no arteriosclerosis. All are young individuals.

Fig. 2.—Pulse-tracing, Case 5. A typical tracing in manic cases

CASE 4.—S., a young woman, is excited, restless, talkative and distractible, but varies in the disturbed condition. Four readings are given:

Systolic pressure	Diastolic pressure
118	78
112	74
130	80
120	80

The first and second readings taken were lower. The first was taken during a very quiet period and the second followed a sedative pack. It would seem to indicate in the first instance a decrease in all the features of the manic tracing as restlessness diminished. In the second instance the sedative pack relaxed the general muscular tone. The other two tracings taken in this case show all the previously described features.

CASE 5.—S., a young man, has had a previous attack; is flighty, talkative and combative; physically, negative. The systolic pressure is 154, diastolic pressure 96 (Fig. 2). This is one of the most typical tracings I have illustrating manic features.

CASE 6.—T., aged 34, has mania of three months' duration. The patient has had a previous attack; is talkative, quarrelsome, expansive and irritable. The condition physically is negative. The systolic pressure is 160, diastolic pressure 108.

CASE 7.—O., aged 24, has had a previous attack of mania. The patient formerly had pressure of speech and activity, but now is emaciated; anemic, has a poor appetite and otherwise is negative physically. At present much less activity is shown. The large amplitude of the wave and early onset of the oscillation were present in the tracing, although the systolic pressure was 138 and the diastolic pressure was 80. The pulse-pressure was 58—not so high as in the more active cases, yet higher than normal and much higher than the pressure was in any of our stuporous cases or in the catatonic who sat next to him.

NON-PRODUCTIVE MANIAS

CASE 8.—W., aged 27, has mania of one and one-half years' duration. The patient is irritable but careless; is interested and happy but non-productive. There are no physical findings. Four readings are given:

Systolic pressure	Diastolic pressure
104	78
100	80
134	82
110	74

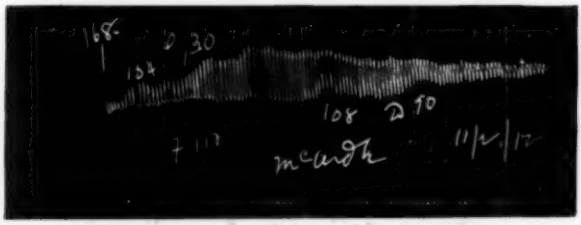


Fig. 3.—Pulse-tracing, Case 9. Persistence of characteristic features of manic states, though less marked in this case.

In one reading the patient was a little active, objecting to the procedure, and the systolic blood-pressure rose to 134. Otherwise the blood-pressure is near normal, although we still find the tendency toward larger amplitude, rapid onset and rapid action in the tracing, all of which were more noticeable at the time she was disturbed.

INTERMISSION IN A CASE OF MANIA

CASE 9.—McA., a young girl, who has recently been excited, is now quiet but not well. The systolic pressure is 130, diastolic pressure 90, pulse-pressure 40, all with a tendency toward a slight rise. The amplitude and other distinguishing features are noticeable (Fig. 3) but are not marked.

RECOVERY FROM MANIC STATE

CASE 10.—K., aged 30, has mania of one year's duration. She is negative physically; is talkative, flighty and active. Three readings are given:

Systolic pressure	Diastolic pressure
130	80
120	80
120	80

As she recovered the manic features in the tracing subsided and the blood-pressure dropped 10 mm.

STUPOROUS CASES

Out of the stuporous cases I have selected two in which there are practically the same age and duration, the same degree of emaciation and the same mental manifestations. They have been selected for their similarity and also for their dissimilarity, in that one patient had arteriosclerosis and kidney lesion, while the other was free from these conditions.

Fig. 4.—Pulse-tracing, Case 11. Small amplitude, slow action. Blood-pressure not above normal.

CASE 11.—N. J., aged 35, is married and has had a psychosis of one and one-half years' duration; is cyanotic, emaciated, stupid, retarded and depressed. There is slight arteriosclerosis. The urine shows a faint trace of albumin. Five readings are given:

Systolic pressure	Diastolic pressure	Pulse-pressure
100	80	20
110	74	36
110	72	38
126	98	28
118	88	30

The average systolic pressure is 113, the average diastolic pressure is 83, the average pulse-pressure is 30.

Fig. 5.—Pulse-tracing, Case 12. Small amplitude, slow action, low blood-pressure.

CASE 12.—A. F., single, aged 37. Psychosis of one year's duration. Ideas of unworthiness and commission of unpardonable sins are present. The patient is retarded, cyanotic and emaciated. Six readings are given:

Systolic pressure	Diastolic pressure	Pulse-pressure
90	60	30
90	60	30
78	64	14
90	64	20
92	58	34
82	60	22

The average systolic pressure is 87, diastolic pressure 62, pulse-pressure 26.

In the case with the arteriosclerosis and kidney lesion (Fig. 4) the blood-pressure is higher than in the other case (Fig. 5). Both show small amplitude and slow action. The blood-pressure is quite low in the second case. In the first case it is not above normal.

DEPRESSION CASES

CASE 13.—K., aged 54, a horse-shoer and a native of Illinois, has insanity of a few months' duration; is worried, hopeless, suicidal, depressed and slightly retarded. The heart and arteries are negative in condition. Albuminuria is present. Five readings are given:

Systolic pressure	Diastolic pressure
166	130
168	126
200	160
182	120
142	90

The pulse-pressure varies from 40 to 50.

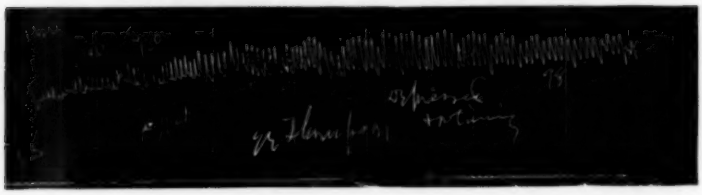


Fig. 6.—Pulse-tracing, Case 14. Effect of muscular resistance.

The tracing which was taken in this case lacks the rapid onset seen in the tracing of the manic. The onset was more tedious and long drawn out. The amplitude varies as the blood-pressure varies, being greater in those tracings which exhibit the higher pressures. Here the factor seems to be the kidney lesion, which varies in its effect on the circulation. In no tracing made in these cases, however, is there shown the amplitude or rapidity seen in the manic case tracings. The mental condition seems to be the same from day to day. We will compare this case with one without arterial or kidney trouble.

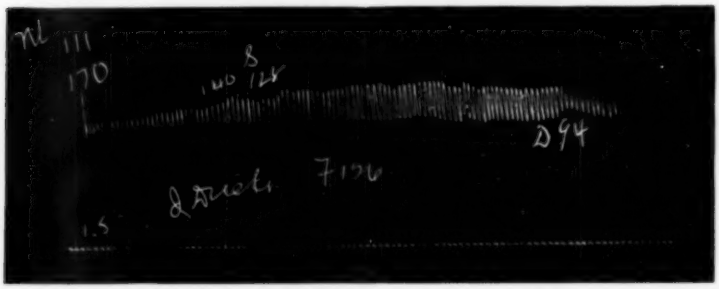


Fig. 7.—Pulse-tracing, Case 15. Low amplitude, slow action and slow approach. Contrast with manic conditions.

CASE 14.—T., aged 34, has insanity of one year's duration. He has had previous attacks; is retarded, fearful, remorseful and subject to spells of agitation. The systolic pressure is 130, diastolic pressure 78. At the beginning of the experiment he was cooperative but became resistive and agitated, and with this the diastole became prolonged. This gives an increase in pulse-pressure, though the blood-pressure is low. The approach of the needle to the systolic reading is slow, the amplitude is small. This tracing (Fig. 6) also illustrates the effect of muscular resistance on the readings.

CASE 15.—D., aged 40, is depressed, hopeless, slightly retarded and working. The physical condition is negative. The average systolic pressure is 117, diastolic pressure 86, pulse-pressure 31. The blood-pressure is low, as is also the pulse-pressure. The same kind of tracing (Fig. 7) was obtained as in other cases of the same type, namely, low amplitude, slow action and slow approach. This is to be contrasted with the features in the previously described tracing in manic cases.

Fig. 8.—Pulse-tracing. Case 20, showing blood-pressure rise with muscular resistance.

CASE 16.—B., aged 29, has a first attack of insanity of a few months' duration. Ideas of unworthiness and a feeling of insufficiency are present. The patient is in a negative physical condition. The systolic pressure is 106, diastolic pressure 78, pulse-pressure 28. Low pressure, both of blood and pulse, and the same kind of tracing as previously described, were noted.

MELANCHOLIAS

In these cases is seen (Figs. 8 and 9) the effect of muscular resistance in raising the blood-pressure regardless of the condition of the arteries. After such patients become calm the blood-pressure falls nearly to normal if there is no arterial disease, and while the mental state is constant, the

Fig. 9.—Pulse-tracing, Case 20, showing blood-pressure rise with muscular resistance.

blood-pressure varies, depending more on the variation of peripheral resistance than on any change in mood. These patients are in middle life and a higher average might be expected, other conditions being normal.

In those cases in which arteriosclerosis is present the average blood and pulse-pressures are higher than in the cases in which arteriosclerosis does not exist.

CASE 17.—C., aged 48, has melancholia of three years' duration. The patient had a previous attack; is poorly nourished, tuberculous, agitated at times, deteriorated, depressed and stupid; a marked arteriosclerosis exists.

This case really belongs to those classified as stuporous depressions, though the blood-pressure and pulse-pressure were slightly high for a stuporous case. Four readings are given:

Systolic pressure	Diastolic pressure	Pulse-pressure
110	86	24
114	84	30
126	88	38
132	90	42

This could be accounted for by the arteriosclerosis. The amplitude of the wave was a little larger on this account, but far short of the large amplitudes seen in the tracings of manic cases.

CASE 18.—C-2, aged 56, has melancholia of a duration of one year. The patient has ideas of persecution and hallucinations. Though at first she was agitated, restless, and apprehensive there was later a deterioration to fussing, upbraiding and crying, though the condition was one of tolerance. Five readings are given:

Systolic pressure	Diastolic pressure	Pulse-pressure
136	88	48
112	80	32
110	80	30
130	90	40
120	88	32

In this case the tracings show moderately low blood-pressure and pulse-pressure which do not rise except when the patient resists, which occurred when the readings were taking. Nothing distinctive was shown in these tracings.

CASE 19.—S., aged 55, has melancholia of two years' duration; has delusions of persecution, is suicidal, distressed and has periodically marked anxiety. The physical condition is negative. Seven readings are shown:

Systolic pressure	Diastolic pressure	Pulse-pressure
130	70	60
110	70	40
112	70	42
120	70	50
120	74	46
130	80	50
120	90	30

The first and sixth readings in which the blood- and pulse-pressure are 130 and 60, 130 and 50, respectively, show the effect of her resistance. This does not reach the height seen in tracings of manic or arteriosclerotic cases, and distinctly lacks the special features noted in the tracings of the former.

CASE 20.—M., aged 45, who was formerly suicidal and anxious, is now empty and mildly resistive at times. A slight arteriosclerosis exists. Six readings are given:

Systolic pressure	Diastolic pressure	Pulse-pressure
160	100	60
116	80	36
134	80	54
122	88	34
140	90	50
110	76	34

The first reading shows the effect of muscular resistance in raising blood-pressure and pulse-pressure (Figs. 8 and 9).

CASE 21.—M., aged 73, is agitated, unclean; has feeling of unworthiness, is excited and apprehensive. The ideas are all present but the affect is gone. Arteriosclerosis exists. Six readings are given:

Systolic pressure	Diastolic pressure	Pulse-pressure
160	80	80
140	80	60
170	70	100
140	88	52
130	80	50
160	80	80

Though the mental condition did not change, the blood-pressure did, owing to the variation in the peripheral resistance in the sclerosed arteries. This shows the value of many readings in any case. One reading was 170, another 130 for systolic blood-pressure.

CASE 22.—B., has melancholia of one and one-half years' duration; is depressed, has feeling of unworthiness, is apprehensive and suicidal. The arteries are palpable. Deteriorated to an empty condition. Four readings are given:

Systolic pressure	Diastolic pressure	Pulse-pressure
160	100	60
140	110	39
146	102	44
160	110	50

The patient was neither agitated nor resistive. The high blood- and pulse-pressures are due to the sclerosis of the arteries.

CONCLUSIONS

1. Cases of mania show typical features in the tracings which are taken, and these are probably due to an altered state of vasomotor tonicity in keeping with the other motor phenomena in these cases. These features are large amplitude of the curve, rapid action and short interval between beginning of oscillation and full amplitude. The blood and pulse-pressures are raised and are more marked, as are the other features just spoken of, as the restlessness becomes more marked. All decrease as the patients recover. In non-productive cases these features are much diminished, but still demonstrable. Arteriosclerosis existing in manics raises the blood- and pulse-pressures still higher. The usual features of the manic tracing are as apparent here as in cases without arterio-sclerosis, and the larger amplitude, rapid action and early onset in the tracing are not features of the sclerosis, but of the manic state. In cases of sclerosis occurring in other types of psychosis these features are lacking.

2. In stuporous cases the blood- and pulse-pressure are quite low. The amplitude is quite small and action is slow and tedious. This seems to indicate a condition of relaxation of the blood-vessels, in keeping with the other phenomena exhibited.

3. In depressed cases the blood- and pulse-pressures are lower than in the manic states, but higher than in the stuporous states. The amplitude of the wave, its onset and rapidity is less than in the manic states, but higher than in the stuporous states. Any rise in any of these things is

due either to muscular resistance or physical disease and not to any state of mind.

4. In melancholia the average blood-pressure is relatively high, for the patients are among those in middle life. The blood-pressure and pulse-pressure are near normal as long as there is no muscular resistance, and no other factor, such as arteriosclerosis, to produce a rise. The tracings show nothing peculiar, except when these factors are added, and then blood-pressure is raised; the amplitude of the wave is also slightly increased.

5. By taking many readings it may be possible to attribute high blood- and pulse-pressures in some cases to the varying effect of arterial and kidney disease on peripheral resistance rather than to the effect of the mental condition. In other cases resistance on the part of the patient is a factor in raising the pressure — a fact which might be overlooked had only one reading been taken at such a time.

AGE INCIDENCE IN CARCINOMA*

CARL VERNON WELLER, M.D.
ANN ARBOR, MICH.

The progressive increase in the incidence of carcinoma as the middle period of life is approached and reached has long been recognized. Most writers have contented themselves with a statement of this fact, leaving the reader to understand that even in advanced years the progressive increase is maintained. For instance, Bashford[1] says: "Carcinoma appears in the human subject with increasing frequency as life advances." He recognizes, however, that the carcinoma incidence in those organs which undergo a definite phase of involution long before senile changes in the organism as a whole are marked, is highest during such involutionary processes. This he bases on statistics of breast and uterine cancer.

Senn[2] gives a suggestion that in extreme old age the cancer incidence is decreased, as the following quotation shows: "Carcinoma manifests a predilection for the conditions incident to senile marasmus, occurring most frequently in persons between 50 and 70 years of age."

It was in the hope of throwing additional light on this and allied problems that the analysis here presented of over 1,100 microscopically verified cases of carcinoma was undertaken.

Several varieties of cancer statistics have been made use of in similar investigations. Such are mortality returns, cancer censuses and hospital and laboratory reports. From the standpoint of a study of age-incidence the first-named fails, in that mortality returns give only the age of the victim when the disease has overpowered him; the cancer census fails in that the report is encumbered with unverified cases; while the greatest objection that can be raised against hospital and laboratory reports is that they are not derived from a representative fraction of the total population.

Our material consists of consecutive cases of carcinoma of known age, diagnosed in the Pathological Laboratory of the University of Michigan between the years 1895 and 1913. This material possesses the following advantages: 1. Every case has been microscopically verified. 2. The age of the patient is obtained at a much earlier stage than in mortality records and thus the actual age-incidence is more nearly represented. 3. The cases are derived from a fairly representative fraction of the total population. The larger part are from the university hospitals, which receive

* From the Department of Pathology, University of Michigan.
* Submitted for publication August 8.
1. Bashford: Imp. Cancer Research Fund, 1905, No. 2, p. 32.
2. Senn: Pathology and Surgical Treatment of Tumors, p. 66.

patients from the entire state, and, to a lesser degree, from neighboring states. Because of this fact, and also because they are not charity hospitals, the patients are much more representative of the general population than is ordinarily the case.

The figures for the age distribution of the total population are derived from the United States census report for 1900.[3] This census report was chosen as being more nearly at the midpoint of the period during which the cases occurred than was the census report of 1910. Since more than 90 per cent. of the cases reported are from Michigan, the figures for that state alone were considered. The error here is negligible for the varia-

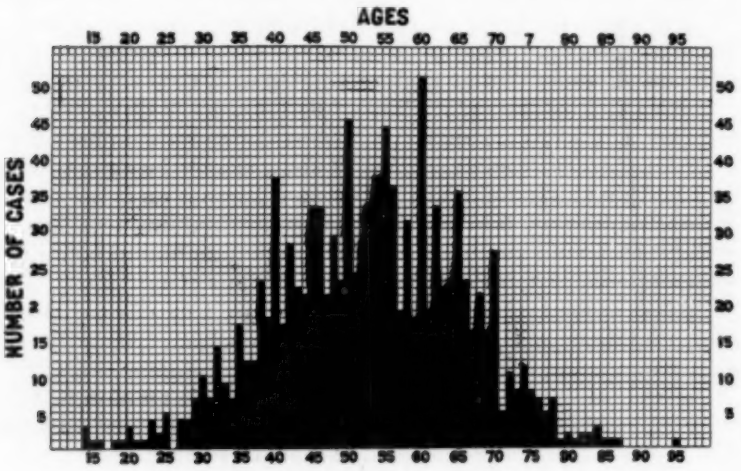


Chart 1

tions in age distribution over a period of a few years, or in neighboring states as compared to Michigan in 1900, are very slight.

The yearly distribution of the total 1,106 cases is shown by Chart 1. The greatest number for any year is found at the age of 60 with fifty-one cases. The error of approximate answers is well shown by the disproportion in the number of cases for consecutive years. The multiples of five are favored by patients in giving their ages. The even ages show a slight preponderance over the odd. This disproportion is found in some degree in all census statistics.[4] In order to avoid this source of error all analyses have been based on five-year periods having the multiple of five as the middle year in the group. Thus the age period 58 to 62 will presumably represent an accurate grouping of all of the cases given as of age 60,

3. Report, Twelfth Census, U. S., 1900, ii, Part 2, 52.
4. Report, Twelfth Census, U. S., 1900, ii, Part 2, 35.

while if the usual quinquennial groups were used, the group 60 to 64 would include many cases which in reality belong in the preceding period.

The percentage of the total population living during each age period and the percentage of the total number of cases of carcinoma for each age period are shown by the two curves of Chart 2. The carcinoma curve lies below the population curve up to age 37. Here the curves cross. At this point the ratio between the two curves is unity, and it may be interpreted as being the point at which the number of cases of carcinoma is the same as it would be were carcinoma uniformly distributed throughout the total population. Beyond this point the ascent of the curve of

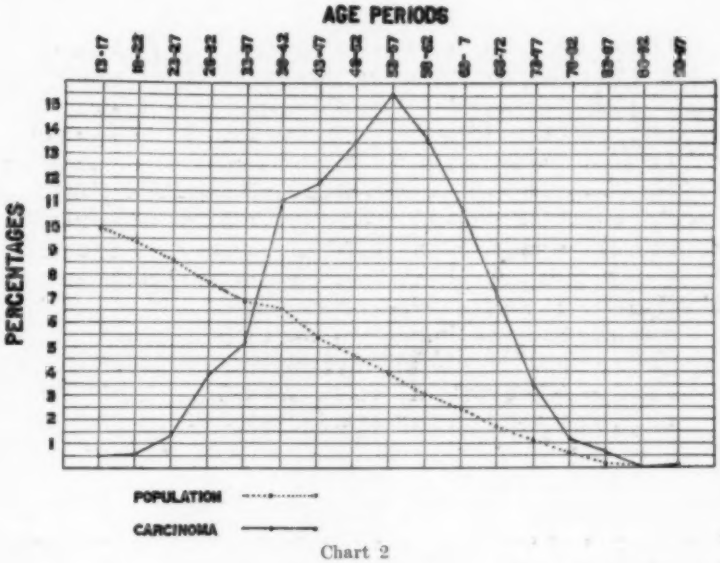


Chart 2

carcinoma percentages is very rapid and the highest point is reached in the age period 53 to 57.

This does not mean that the carcinoma incidence is greatest during this age period, for the decreasing population leaves fewer individuals to be attacked. This point has been further elucidated by computing a series of ratios between the percentages previously used, that is,

$$\text{Ratio for age period N} = \frac{\text{percentage of carcinoma for age period N}}{\text{percentage of population for age period N}}$$

These ratios are plotted in the curve of Chart 3. As previously mentioned, the value is less than one up to age 37 and greater than one thereafter. The apex of the curve is at the age period 58 to 62, and this, therefore, represents the age period of greatest carcinoma incidence.

From 58 to 62 there is a definite fall in the curve, which becomes most marked after 66 to 72. The abrupt rise after 78 to 82 is meaningless, since it is determined by too small a number of cases (eight) to have any value. One must therefore conclude that after 70 years of age there is a decided diminution in the incidence of carcinoma.

Chart 4 shows graphically the age distribution of our cases of carcinoma divided according to sex. The cases in males totaled 495; in females 611. It is noticeable that the curve for female cases rises on the average ten years before that of males, reaches its apex ten years earlier and falls on the average five years ahead of the curve for males. Since,

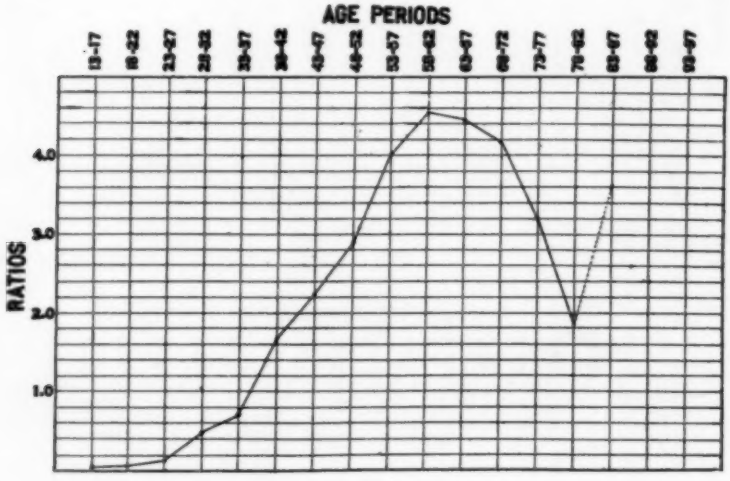


Chart 3

however, the total number of cases differs with the sexes, and the population totals for the sexes are also unlike in their age distribution, no definite conclusions can be drawn from the data presented by Chart 4. It has, therefore, been necessary to construct ratios on the same general plan as before, that is,

$$\text{Ratio for males, age period N} = \frac{\text{percentage of carcinoma in males at age period N}}{\text{percentage of male population at age period N}}$$

and

$$\text{Ratio for females, age period N} = \frac{\text{percentage of carcinoma in females at age period N}}{\text{percentage of female population at age period N}}$$

By plotting the two series of ratios a graphic representation of the incidence of carcinoma in each sex and at each age period is obtained and a direct comparison is possible (Chart 5). The ratio for females reaches unity about the age 33, while for males this point is not reached until age 39. Among females the incidence of carcinoma reaches its height in

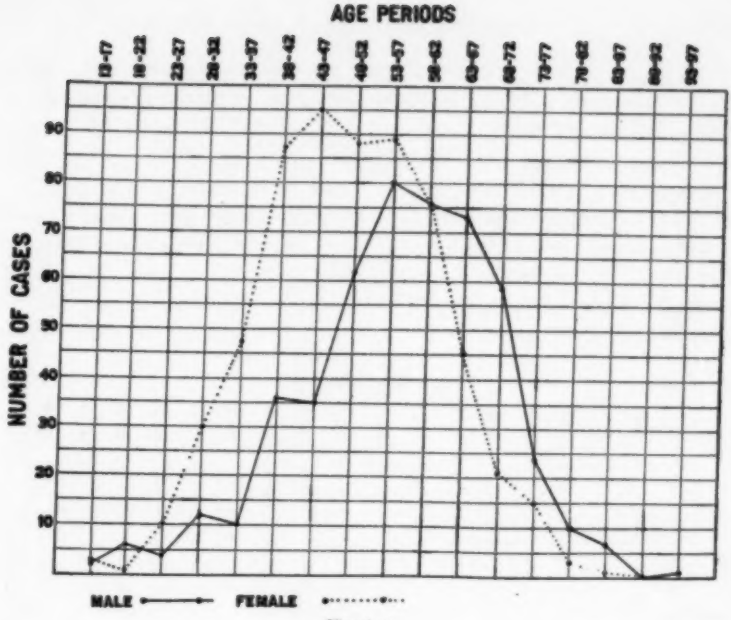


Chart 4

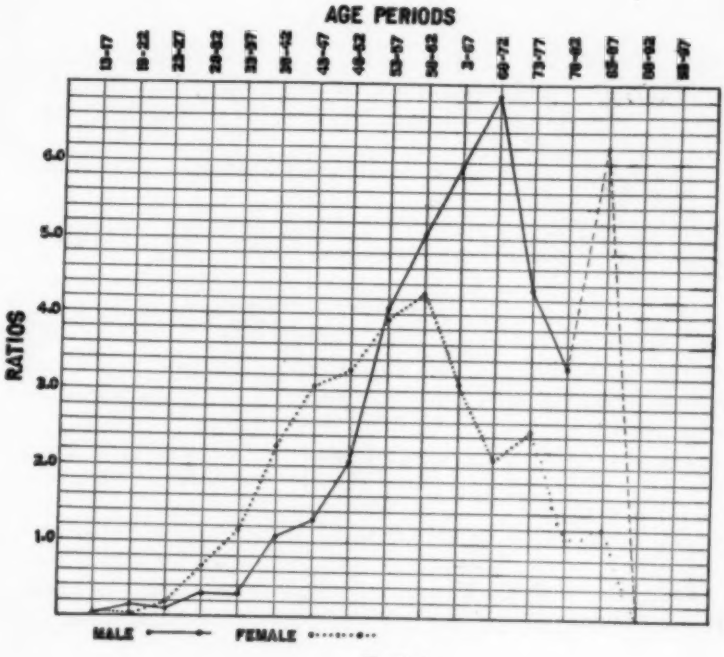


Chart 5

the age period 58 to 62, while carcinoma is most frequently recognized in males at 68 to 72. These latter figures are made somewhat higher than they should be by the cases of basal-celled carcinoma which, occurring most frequently in males, are often diagnosed rather late in life, though they may have been of many years standing. But, even with due allowance for this source of error, it is evident that in the female the wave of carcinoma incidence traverses its cycle five to ten years earlier than in males. The sharp decrease in both curves after the crest of the curve is reached holds good for each sex quite as well as it did in the combined statistics of Chart 3.

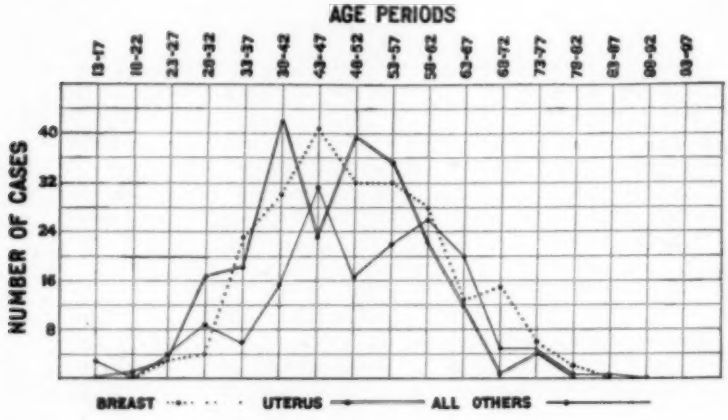


Chart 6

In order to discover what elements in the curve of female cases of carcinoma were responsible for causing it to precede the male curve in all its phases, the female cases were divided into three groups according to the location of the carcinoma.

Breast	229 cases.
Uterus	219 cases.
All others	165 cases.

The age distribution of each of these three types is shown by Chart 6, and in Chart 7 the percentages of the total number of cases in each group occurring during each age period are plotted. It is found that between 33 and 43 the percentages of cases in which carcinoma of the breast and uterus occurs are much higher than that of the group which is designated "all others." These two sources, comprising more than two-thirds of all our cases of carcinoma in women, are therefore very powerful factors in determining the earlier cycle in the general curve for women.

The form of the curves in this chart becomes less regular because of the smaller number of cases being considered. There is one irregularity,

however, which while it may be due to the smaller number of cases, may also have a much deeper significance. In the curves which indicate cases in which carcinoma of the uterus occurs, we find:

42 cases, or 19.4 per cent., at age period 38 to 42
23 cases, or 10.6 per cent., at age period 43 to 47.
39 cases, or 18.0 per cent., at age period 48 to 52.

The peculiar drop just at the menopause period, 43 to 47, may find its explanation in the fact that metrorrhagia at this time is usually considered by the patient as but a phenomenon of the climacteric, and therefore unworthy to be investigated.

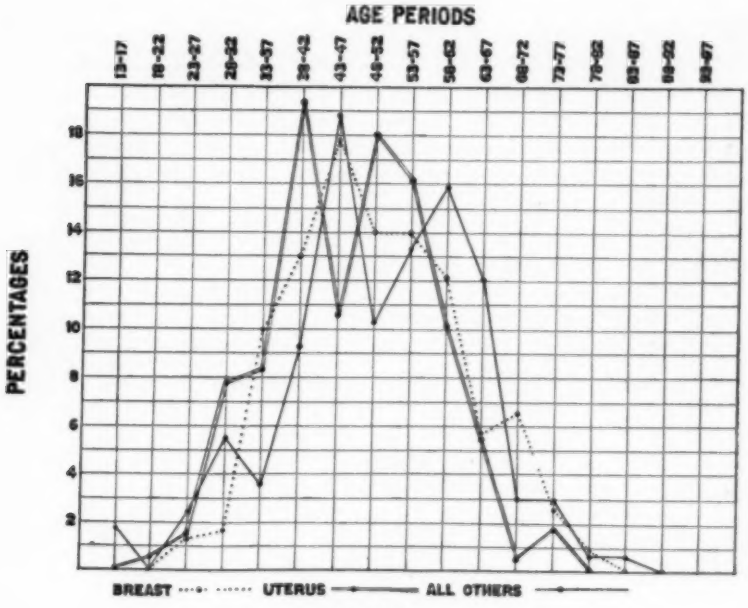


Chart 7

CONCLUSIONS

The analysis here given indicates that:

1. The incidence of carcinoma is greatest at the age period 58 to 62.

2. After this period the carcinoma incidence decreases.

3. Carcinoma incidence in women runs a definite cycle which is roughly parallel to that in men, but precedes it by five to ten years.

4. The earlier age for the phases of the curve for women is largely due to the earlier appearance of carcinoma of the breast and uterus as compared to other varieties of carcinoma.

HEREDITY WITH REFERENCE TO CARCINOMA

AS SHOWN BY THE STUDY OF THE CASES EXAMINED IN THE PATHOLOGICAL LABORATORY OF THE UNIVERSITY OF MICHIGAN, 1895-1913 *

ALDRED SCOTT WARTHIN, M.D.
ANN ARBOR, MICH.

The statistical study of carcinoma is regarded by many writers as having been carried as far as it can be profitable; and certainly but little that is new has been gained through this method during the last decade. Nevertheless, its possibilities have not been exhausted; and it is highly desirable that the whole neoplasm problem in all of its aspects be attacked again from the statistical standpoint, though in a somewhat different way. Practically all of the old statistical studies of neoplasms, particularly those of carcinoma, were based on mortality reports; or if not on these, on morbidity reports based on clinical diagnoses. In very few instances only has the statistical study been carried out on the basis of the records of a diagnostic pathological laboratory. Statistics of neoplasm from such a source must be of infinitely greater value than those founded on mortality statistics. In the records of the diagnostic laboratory the diagnosis is based on the histological examination, and the percentage of error is reduced to a minimum. In the mortality statistics, on the other hand, the diagnoses are chiefly clinical, and consequently subject to the wide error inherent in the clinical diagnosis of "tumor," neoplasm, "cancer" and the like. Moreover, the material coming to the diagnostic laboratory is usually seen from two to five years earlier than the mortality age. In studies relating to the age-incidence of any form of neoplasm it is evident that the records of the pathological laboratory for that neoplasm will be much more trustworthy than the mortality statistics. It is also possible many times in the diagnostic laboratory to follow the course of a neoplasm over a definite period, so that important practical knowledge may be gained as to rate of growth, recurrence, healing, metastases, etc.

The following study of the influence of heredity on carcinoma is taken from the records of the pathological laboratory of the University of Michigan during the years of my service from 1895 to 1913. During this period 3,600 cases of neoplasm of all varieties have been studied, either in material taken for practical diagnosis or obtained by necropsy. Of these 3,600 cases, some 1,600 were cases of carcinoma, as was shown by the microscopic diagnosis. Practically every variety of carcinoma

* Submitted for publication Aug. 8, 1913.

described in the literature, and a few others, as well, are to be found in this material; and the same is true of the other forms of neoplasm. While carcinomas of the breast, uterus and lip form the greater part of the cases examined, all other localizations are represented. Another great advantage is that about 90 per cent. of the material was derived from the general population of the state of Michigan. The fact that the university hospital is a state hospital and not a charity institution, gives it a much more representative population than would be found in the charity hospitals of the greater cities.

The difficulty of obtaining a family history from patients in charity hospitals is well known. Few of them know the cause of death in their grandparents or in other members of that generation. The same thing is true, though to a less extent, of the patients from whom the material used in this study was obtained. Therefore what worth this study may have must be positive; the negative findings cannot be taken as absolute. In about six hundred cases of carcinoma no family history was obtainable; in the majority of those in which a family history was obtainable the details are meager. In spite of these handicaps, in a surprisingly large number of cases a family history of carcinoma is given — about 15 per cent. of all in which the family history is obtainable.

The existence of a family susceptibility to carcinoma, denied by many writers, affirmed by others, has again become, as a result of the studies of endemic carcinoma in mice, a question exciting lively interest. These investigations appear to show that certain family strains in mice do not develop spontaneous tumors, and that, further, such strains are resistant to tumor transplantation from other mice. Other family strains show a high frequency of spontaneous tumors, and in such strains transplantation may be successfully carried out. It would appear then, that the latter strains possess a familial predisposition or susceptibility to the development of neoplasm, and that intrinsic or inherited factors play some part in the development of neoplasms. Bashford denies this, and Tyzzer, who carried out breeding experiments with animals representing different strains, was unable to find that the transmission of the predisposition to neoplasm followed the laws of heredity.

On the other hand, a family predisposition in the human species to certain forms of neoplasm has long been recognized, particularly in the case of certain benign neoplasms (fibroma, lipoma, chondroma, osteoma, angioma, leiomyofibroma, glioma, neurofibroma, papilloma, adenoma and cyst-adenoma), and to a less marked degree in the case of certain forms of sarcoma and carcinoma. While the existence of such a predisposition is generally accepted by text-books on pathology, no good statistical studies exist of the hereditary occurrence of these neoplasms.

Cancer surveys of living patients, as conducted in Germany some years ago, have given little information concerning the family occurrence of neoplasms, chiefly for the reason that it is practically impossible to trace the members of a given family through more than one or two generations, and unless all of the members of the family for several generations can be considered, no accurate conclusions can be drawn. The difficulties attendant on such a statistical study are very great; and except in rare cases complete family records of cancer incidence are not found in the literature. One of the most striking is Broca's case,[1] also quoted by Levin, who criticizes the method of investigation, because no comparison was made between the number of the affected and unaffected members in each generation. If the total number of ascendants is not considered, and only those affected by carcinoma picked out, a family may appear to be a cancer family when in reality the proportion of cancerous to non-cancerous members is very small. As Levin states, Broca's family might give very different results if studied from the standpoint of the history of all its members.

Levin, to my knowledge, is the only one who has attempted a statistical study of heredity in cancer from the standpoint of an entire family history. He collected data[2] from five families, in two families fairly complete and in three fragmentary. In the families studied he noted that they were characterized on the whole by the occurrence of cancer of the intestines in the males, and cancer of the breast in the females. His charts show that the incidence of cancer in these families is not greater numerically than would be found in the population as a whole, but that the occurrence of "cancerous fraternities" (a fraternity in which one or more members suffer from cancer, with a history of cancer either on the maternal or paternal side, or both) speaks for some influence of heredity on cancer. In other words, a cancerous fraternity must mean the union of two germ-plasms, each of which is characterized by the presence of germ-cells that are non-resistant to cancer.

In my material of 3,600 cases of neoplasm examined pathologically, there were 1,600 cases of carcinoma. About one thousand of these gave fairly good family histories with the ages of the members. A smaller number (30 per cent.) gave detailed histories. From this number are taken the families which show multiple occurrence of carcinoma. In many of these all of the members of the family for three generations are given; in others, the records are incomplete. Four families give complete records of the descendants of the cancerous grandparent. The incidence of cancer in these families is so striking that it can be interpreted as showing an inherited susceptibility to cancer.

1. Broca: Quoted by Wolf, Die Lehre von den Krebskrankheiten, Jena, 1907; Williams, p. 369.
2. Levin: Ztschr. f. Krebsforsch., 1912, ix, S. A., p. 5.

CANCER FAMILIES

FAMILY G. (Chart 1).—In this family a fairly complete survey was made of the two generations derived from a cancerous grandfather with a traditionary history of cancer in his line and a grandmother with a normal family history. From these there were ten children, five males and five females. Two of the daughters died of cancer of the uterus at 55 and 40 years, while two sons died at 42 of cancer of the stomach, and a third one at 45 of cancer of the abdomen. All five of these individuals were married to normal partners without a family history of cancer, and all had issue, as follows: Oldest daughter who died at 55 of cancer of the uterus, had ten children: one daughter operated on at 42 for "cancer" of the uterus and still living; another daughter operated on at 22 for uterine tumor and bilateral dermoids of ovary, and still living. The remaining eight children are all living and well, only two being over 40 years of age. The second daughter, who died at 40 of cancer of the uterus, had four children, two sons and two

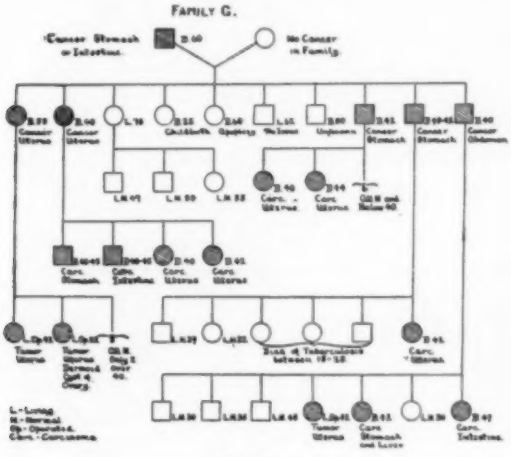


Chart 1

daughters all dying of cancer, the two sons of cancer of the stomach and intestine, and the two daughters of carcinoma of the uterus. The third daughter, living and well at the age of 75, has three normal children living at the ages of 47, 50 and 55. Four children had no living issue. The eighth child, a son, died at 42 of cancer of the stomach. His wife was of normal family history. They had eight children, of whom two daughters have died of cancer of the uterus at 40 and 44 years, while the remaining six are all living and well below the age of 40. The ninth child, a son, died of cancer of the stomach when between 40 and 42 years of age. He left six children from a marriage contracted with a woman of non-cancerous family history. One daughter died at 42 of cancer of the uterus, three children died of tuberculosis between the ages of 18 and 25 years, while two others are living and well at the ages of 32 and 29 years. The tenth son died at 45 of cancer of the abdomen, most probably primary in the stomach. He left, from a marriage contracted with a woman of non-cancerous family, seven children, of whom one died at 42 of cancer of the stomach and liver, another at 47 of cancer of the intestine, while a third was operated on at 42 for tumor ("cancer") of the uterus, and still lives in apparent good health. Four others are living and normal at the ages of 45, 35, 30 and 30.

Of the forty-eight descendants of the cancerous grandfather seventeen have died or been operated on for "cancer." The preponderance of carcinoma of the uterus (ten cases) and of the stomach (seven cases) is very striking in the family history.

FAMILY F. (Chart 2).—In this family the maternal grandmother died of tumor. Her non-cancerous brother had two children, both of whom died of "cancer." Her only son died at 61 of dropsy. He married a woman who had two brothers who died of cancer of the stomach. She herself died of Bright's disease at 75. Her

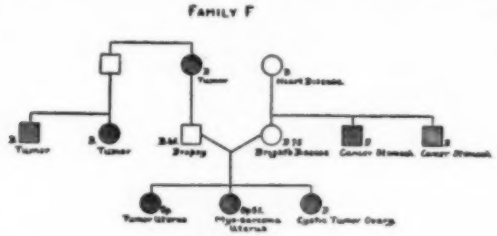


Chart 2

mother died of heart disease. The three daughters of this pair who show a double family history of susceptibility to cancer all had neoplasms; the oldest was operated on for tumor ("cancer") of the uterus and is still living; the second was operated on at 51 for myosarcoma of uterus, while the third daughter died of cystic tumor of the ovary. In this family history the preponderance of stomach and uterine neoplasms is also shown.

FAMILY P. (Chart 3).—The paternal grandfather had a nephew who died of cancer of the lip. In the first filial generation there was one daughter who died at 35 of cancer of the lip and a son who died at 86 of cancer of the scalp and cervical lymph-nodes. This son married a non-cancerous woman whose only sister

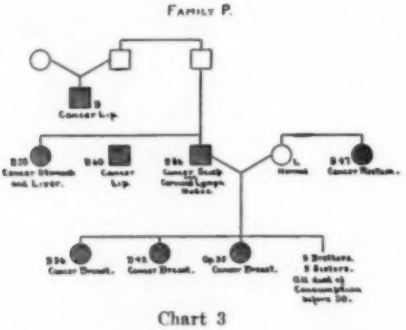


Chart 3

had died at 47 of cancer of the rectum. From this union thirteen children, ten of whom (five brothers and five sisters) all died of pulmonary tuberculosis before the age of 30, while three remaining daughters had carcinoma of the breast, two dying at the ages of 36 and 42, and one operated on at 35.

FAMILY S. (Chart 4).—The paternal great-grandfather died at about 70 of cancer of the stomach. His only son died at about 60 of cancer of the stomach, having married a woman who died at about 50 of cancer of the breast. They had six children, all of whom died of cancer; two daughters died at 80 and 60 of cancer of the breast, and another at 60 of multiple carcinoma of the breast, bladder and

rectum. Two sons died of cancer of the stomach at the ages of 75 and 40, the third son dying of cancer of some internal organ, most probably the stomach. Only one son had issue, by marriage with a normal line. The only child died at 36 of cancer of the uterus. Of the eight descendants of the cancerous great-grandfather all died of cancer. As in Family P., the occurrence of carcinoma in both paternal and maternal lines apparently strengthens the susceptibility, both families becoming extinct.

CANCEROUS FRATERNITIES

These are of much more frequent occurrence in the case-histories of carcinoma (Charts 5, 6, 7, 8, 9) than the striking family histories given above show. The reasons for this are obvious: Few individuals of the

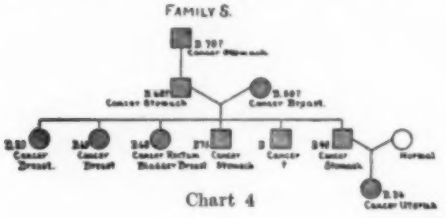


Chart 4

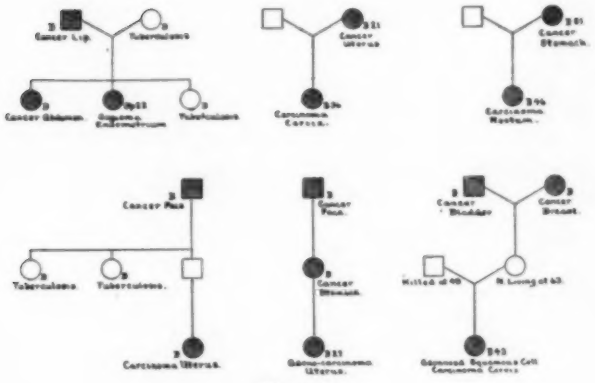


Chart 5

general run of the American population know anything about their family history except for the immediate members. By far the majority do not know the cause of death of their grandparents. Twenty-nine cancerous fraternities are selected as representative of our case-histories. Two generations only are represented in the majority, but in some of them three generations, and in one instance, four generations are shown. The normal members of the second and third generations are also given, so that the proportion of cancerous to non-cancerous individuals in two generations is exact. The majority of the cancerous fraternities occur in small families, and in many cases the patient from whom the material

examined came represented the end of the family line. In several instances all the members of the small family are cancerous. The charts explain themselves. The most striking thing shown, aside from the susceptibility of the family group, is the great prominence of tuberculosis

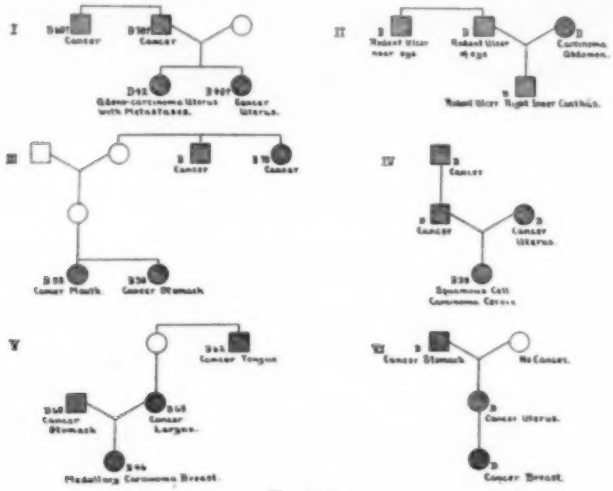


Chart 6

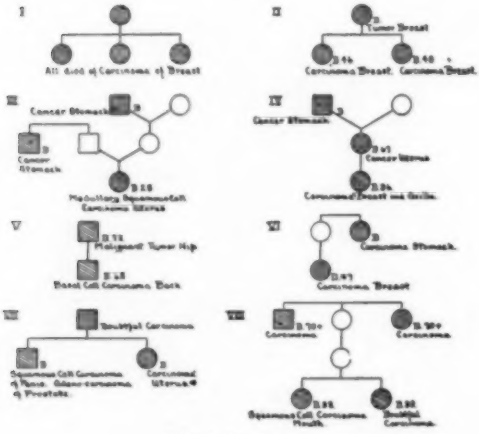


Chart 7

as the most commonly associated disease of the cancer stock. The two susceptibilities seem to run together, at least, in so far as our family histories are concerned. A tuberculous ancestry is not infrequently found in families where there is no family history of cancer back of the present

generation. Next to tuberculosis the diseases most commonly associated with carcinoma are cardiac and renal disease.

It will be noted that the uterus, breast, gastro-intestinal tract and mouth are the parts of the body most frequently involved in the case of these family cancers. Cancer of the lip and rodent ulcer of the face show also a tendency to family occurrence.

The study of a large number of cases of carcinoma yields isolated but striking examples of a marked family occurrence through several generations; and a much more frequent family group or "cancerous fraternity" occurrence. From such histories it is hardly possible to draw any other conclusion than that a definite cancer susceptibility exists in certain families. The great frequency of the association of cancer with tubercu-

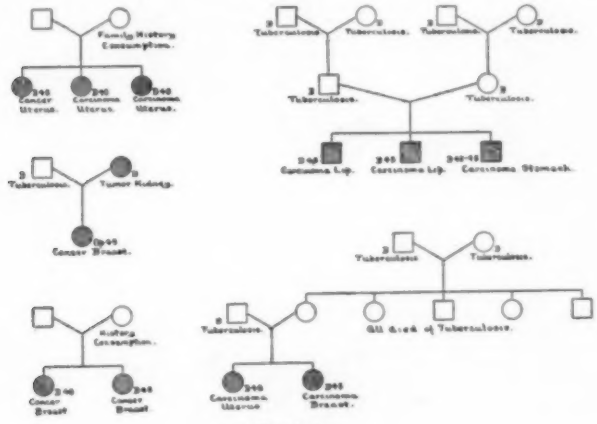


Chart 8

losis might be taken as an evidence of a general weakened resistance on the part of the family lines; and this conclusion is supported by the extinction of many of these lines through a lessened fertility.

In the study of all of our neoplasm material a family susceptibility is occasionally shown in the case of angioma, lymphangioma, fibroma, neurofibroma, lipoma, myofibroma of uterus, adenoma of breast and adenoma of thyroid; but extremely rarely in the case of sarcoma.

CONCLUSIONS

1. A marked susceptibility to carcinoma exists in the case of certain family generations and family groups.

2. This susceptibility is frequently associated with a marked susceptibility to tuberculosis, and also with reduced fertility.[3]

3. The striking association of tuberculosis with cancer in certain families has also been noted by Kuthy and Williams, "The Natural History of Cancer," p. 371.

3. The multiple occurrence of carcinoma in a family generation practically always means its occurrence in a preceding generation.

4. The family tendency is usually more marked when carcinoma occurs in both maternal and paternal lines.

5. Family susceptibility to carcinoma is shown particularly in the case of carcinoma of the mouth, lip, breast, stomach, intestines and uterus.

6. In a family showing the occurrence of carcinoma in several generations there is a decided tendency for the neoplasm to develop at an earlier age in the members of the youngest generations. In this case the neoplasm often shows an increased malignancy.

7. Because of the difficulty of obtaining complete family records, the laws of inheritance of carcinoma susceptibility cannot be determined

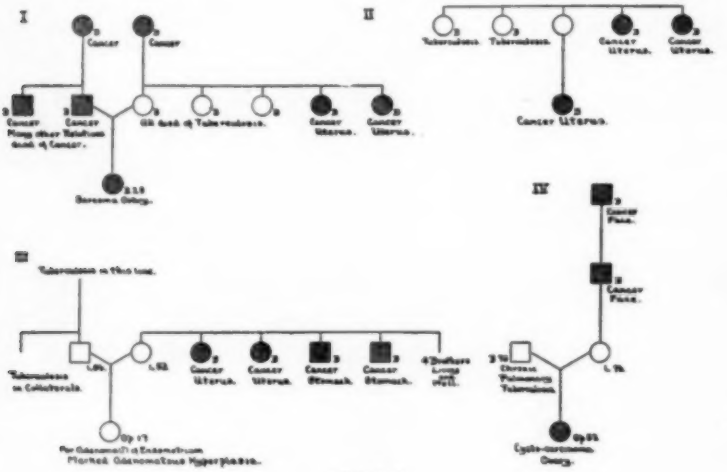


Chart 9

accurately, and it is highly desirable that investigations of large family records should be made relative to the occurrence of carcinoma susceptibility. In Levin's study of cancerous fraternities in connection with the whole family history the percentage of cancerous members in each cancerous fraternity corresponds very closely to the Mendelian percentage of members with recessive unit-characters in a hybrid generation. The same conclusion might be drawn from my cases in certain instances, but it does not seem to me that the data are sufficient for such conclusions. He himself does not consider this conclusion as final. Levin also concludes that resistance to cancer is a dominant character whose absence creates a susceptibility to cancer. While some of my cases show a family history suggesting this, others would indicate a progressive degenerative

inheritance — the running-out of a family line through the gradual development of an inferior stock, particularly as far as resistance to tuberculosis and cancer is concerned.

Levin, as well as Williams,[3] noted the family tendency to specific localization of the cancer, particularly of the uterus in the women, and of the gastro-intestinal tract in the men. This is well shown in my family histories and in some of the cancerous fraternities. Levin concludes that the most important result of his investigation is the fact that it shows the presence of an inherited resistance to cancer growth. I would put it in just the opposite way and say that my observations are important in that they show in certain families an inherited susceptibility to cancer. If the majority of the human race do not show this susceptibility, resistance to cancer is a normal trait of the species. An increased susceptibility becomes, therefore, the abnormal character of importance, and our investigations should be carried along the line of attempting to determine just what lies back of this susceptibility.

3. Williams: The Natural History of Cancer, 1908, p. 364.

A CLINICAL STUDY OF VAGOTONIA*

ARTHUR H. HOPKINS, M.D.
PHILADELPHIA

In 1899 there appeared in the *New York Medical Journal* three admirable articles by Meltzer,[1] in which he deals with the function of inhibition. He speaks of the entire life of the animal as being a delicately adjusted equilibrium between excitation and inhibition and cites instances in which the slightest deviation of the resultant in the nervous mechanism of an organ may lead to the most serious consequences.

A later article[2] by the same author deals with the relation of inhibition to some forms of disease, and though it has paved the way for the more recent work brought forth by Eppinger and Hess[3] in Vienna, only the latter work will be discussed here.

It is my purpose to present a brief summary of their work together with a clinical analysis of a number of cases which I have studied during the last year. At the present time, though I am by no means prepared to support all of their hypotheses, the results are of interest and worthy of further study.

Owing to the difficulty of accurate diagnosis of neuroses, these authors have made a clinical study of such conditions, basing their work chiefly on varying conditions of irritability of the vagus and sympathetic nervous system. They divide the nervous system into the animal and vegetative, the former being represented by all the fibers running to voluntary muscles and sense organs, the latter by fibers supplying smooth muscle organs, as intestines, vessels, ducts of glands, etc.

Recognizing the difficulty in separating either anatomically or physiologically the fibers running to these organs, they attempted and succeeded in making a pharmacological separation, demonstrating the specific action possessed by epinephrin in stimulating the sympathetic system and the selective action of the pilocarpin group, i. e., pilocarpin, atropin, physostigmin and muscarin, for the vagus and vagus extended, or so-called autonomic system. The action of these drugs on the organs

---

* Read before the Section on Medicine of the College of Physicians of Philadelphia, February, 1913, and before the Germantown Branch of the Philadelphia County Medical Society, March, 1913.

* Submitted for publication July 21, 1913.

* From the William Pepper Laboratory of Clinical Medicine. Under the J. Alison Scott Research Fund.

1. Meltzer: New York Med. Jour., May 13, 20, 27, 1899, lxix, 661, 669 and 739.
2. Meltzer: Med. Rec., June 7, 1902, lxi, 881.
3. Eppinger and Hess: Sammlung klinischer Abhandlungen über Pathologie und Therapie der Stoffwechsel und Ernährungsstörungen, 1910.

involved is best demonstrated by Table 1. The accompanying diagram illustrates the autonomic and sympathetic systems anatomically.

Epinephrin being constantly secreted by the adrenals and general chromaffin system, must have a constant stimulating effect on the sympathetic system, and Eppinger and Hess[3] hold that it may be possible that a physiological analogue to epinephrin may exist for the autonomic nervous system. The central nervous system may be the general controller of the antagonistic systems and a disturbance of control, too much or too little irritability, too much or too little nerve tonus of one antagonist may bring about a pathological condition. On this ground they have attempted to clear up the so-called neuroses, studying clinically the irritability of the autonomic system, believing that a fluctuation in tonus or irritability might give an explanation for clinical symptoms.

TABLE 1.—ACTION OF DRUGS

Organs	Pilocarpin	Atropin	Epinephrin
Iris sphincter	Stimulates	Paralyzes	.....
Dilator	.....	.....	Stimulates
Ciliary muscle	Stimulates	Paralyzes	.....
Salivary glands	Stimulates	Paralyzes	Stimulates (?)
Sweat glands	Stimulates	Inhibits	Inhibits
Heart muscle	Inhibits	Stimulates	Stimulates
Vasomotor to head	.....	Contracts (?)	Contracts
Esophagus	Excites	Relaxes	Relaxes
Cardia	Excites	Paralyzes	Paralyzes
Stomach	.....	.....	.....
Tonus	Increases	Diminishes	.....
Peristalsis	Increases	Paralyzes	Paralyzes
Secretion	Increases	Diminshes	Diminishes (?)
Pancreatic secretion	Excites	Inhibits	Inhibits
Bronchi	Excites	Inhibits	Inhibits
Gall Bladder	Contracts	Relaxes	Relaxes
Intestinal Musculature	Excites	Paralyzes	Paralyzes

High tonus in one system is accompanied by increased irritability in the other, and the antagonism must be also a pharmacodynamic one; individuals susceptible to epinephrin being only slightly susceptible to pilocarpin and vice versa. The name *"vagotoniker"* they apply to those constitutions which show functional increased tonus and increased susceptibility to pilocarpin, as well as an insusceptibility to sympathetic stimulation. The entire system or but a single branch may be involved. It finds its expression in latent increase of function and gives in this way to specific irritation a better point of attack than where no increase of tonus exists. Such specific irritation may arise from noxa in the form of bacterial toxins, as during or after acute infections, drugs or the products of deranged metabolism, mechanical irritation and so forth.

SYMPTOMS OF IRRITABLE VAGUS IN RELATION TO INDIVIDUAL ORGANS

Eye.—Ciliary muscle cramp, which is increased by pilocarpin and decreased by atropin; accommodation paralysis as observed after severe infection and finally strabismus may all be explained by vagal irritability.

Salivary Glands.—Salivation due to autonomic irritability may occur in nervous people, in *vagotonikers* and tabetic crises.

Skin.—Sweating is typical, and in crises of many infections, when associated with a slow pulse, suggests increase in tonus of heart vagus. Cold hands and feet suggest stimulation of dilators of peripheral vessels by the autonomic poison.

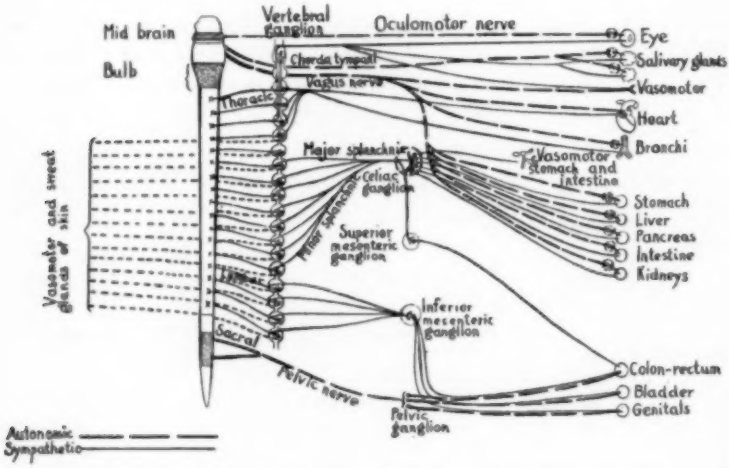


Diagram of the anatomical relations of the autonomic and sympathetic systems (Meyer and Gottlieb, Experimentelle Pharmakologie, 1911, p. 128.)

Heart.—Bradycardia is common in "vagotonikers," especially in the young, and, owing to vagus irritation of toxic or mechanical origin, may be observed in convalescence after acute infections, icterus, brain pressure and many heart poisons.

Nervous block, as described by Hering,[4] is improved by atropin, while some forms of angina pectoris may be attributed to vagal irritability causing narrowing of coronary vessels.

After digitalis the early appearance of bradycardia with gastrointestinal disturbances is proof of special irritability in autonomic area. The combination of atropin with digitalis in such cases is held to be of great therapeutic value.

4. Quoted by Eppinger and Hess; see Note 3.

Lung and Respiration.—Bronchial asthma, which can be produced by peripheral irritation of vagus in animals, these authors assert is a classical example. Being due to a cramp of bronchial musculature, the air in the lung alveoli is not pressed out, the autonomic stimulation leads to muscle cramp with increase of secretion and further hindrance to air going out. Epinephrin checks secretion and atropin tends to relieve the spasm. Strumpell speaks of narrowing of the glottis in this condition, the recurrent laryngeal nerve being related to the autonomic system. Respiratory arhythmia is common.

Stomach.—The vagus influences the form, peristalsis and secretion, and where there exists a physiologically high tonus there may be a great increase in gastric juice and hyperacidity without complaints. Pylorospasm with increased acidity, spasmodic hourglass constriction, cardiac and esophageal spasm, and finally vomiting accompanied by ptyalism in the early stages of pregnancy may all be due to an irritable vagus.

Intestine.—Peristalsis is increased. There may be either diarrhea or constipation.

Blood-Picture.—Eosinophilia occurs especially where the skin, lung or intestinal element is present, and is increased after pilocarpin, often markedly.

Urine.—Rich in phosphates and oxalic acid, especially where there is high gastric acidity; may be slight dysuria.

The so-called vagotonic disposition is described by Eppinger and Hess as follows:

> Often familial in type, this condition is seen most often in young people of nervous tendency who are subject to cold hands and feet, which are frequently bluish and mottled. They have a tendency to swallow when talking, to flush readily and to sweat readily; skin is moist, there is slight internal strabismus, increased power of accommodation, loss of sensation of touch in throat and larynx. Pulse is naturally slow. Marked respiratory fluctuations are present, due to irregularity in contraction of diaphragm. Reflexes increased, dermographism marked. The first complaint may be of gastric or intestinal disturbance.

COMBINATION OF VAGOTONIA WITH OTHER DISEASES

The presence of an irritable vagus, they say, may materially influence the symptoms in the following conditions:

Gastric ulcer or carcinoma, cholangitis, gall-stones, cholecystitis, stone in the kidney tract, tabetic crisis, hyperthyroidism, the menopause, puberty and menstruation, and skin conditions such as urticaria.

The advantage of atropin to combat anaphylaxis and the probable upset nervous mechanism in certain cases of tuberculosis due to destruction of epinephrin in chromaffin system are at least suggestive of a disturbance of this type.

INTERNAL SECRETIONS

This question will not be dealt with beyond calling attention to the fact that epinephrin in the blood has an influence on the internal secretions of the pancreas, which in turn controls production of sugar in the liver. When there is too much epinephrin in the blood the internal secretion from the pancreas is inhibited and the liver can produce sugar excessively.

In cases of vagotonia there is a marked increase in tolerance for grape sugar up to 200 or 300 gm., while in cases of sympathetic neurosis there is a corresponding decrease in tolerance.

The test for vagotonia is as follows (Fleischmann):[5] Before the injection of pilocarpin the average pulse and respiratory rates are taken, the blood-pressure estimated and smears made for a differential blood-count. Pilocarpin, ¼ grain, is then given hypodermically and during the hour the general reaction is observed, as sweating, salivation, lacrimation, increase in nasal secretion, fibrillation, flushing, chills and cold extremities. The pulse and respiratory rates are taken every two or three minutes, the blood-pressure estimated at longer intervals and at the end of one hour more smears are made for a differential blood-count.

A strong general reaction accompanied by a marked increase in eosinophils in the blood, an increase in tolerance for grape-sugar up to 200 or 300 grams, and a cardiorespiratory arhythmia may be interpreted as a positive result.

The following cases, having shown symptoms suggestive of an irritable autonomic system, were tested; Table 2 showing the results:

REPORT OF CASES

CASE 1.—Diagnosis: cardiac extrasystole. Arhythmia.

Capt. R. S., aged 45, presented himself five years ago complaining of attacks of palpitation of the heart or "flutterings" with missed heart-beats. Pulse in interval is slow and feet always cold. Patient is constipated when he stops using tobacco, which he has used to excess. During the attacks of fluttering which are brought on by slight exertion or mental excitement, he voids a large amount of urine.

Aside from the preceding symptoms he has no complaint. Physical examination and history are negative.

CASE 2.—Diagnosis: gastric neurosis.

K. G. F., female, aged 17, complains of regurgitation of food. Three years ago, just preceding onset of menstruation, patient developed regurgitation of food a few minutes after each meal, lasting for about five minutes. This continued for two years, when one year ago the attacks began to persist for about an hour at a time. Patient has a tendency to colds, nasal congestion, and is troubled with much nasal secretion; occasionally vomits freely. She has gaseous eructations toward end of regurgitations.

Examination shows tonsils large, hands and feet always cold and clammy. Roentgen ray shows curvature of spine to left from tenth dorsal to third lumbar

5. The technic of this test is the one used by Dr. P. Fleischmann in the Second Medical Clinic of Krankenhaus Charité, Berlin, to whom I am indebted for it.

TABLE 2.—TEST FOR VAGOTONIA IN TEN CASES*

Before Pilocarpin					After Pilocarpin gr. ¼†									
Case	Pulse	Resp.	Blood Pres.	Eos. Pct.	Pulse	Resp.	Blood Pres.	Eos. Pct.	Saliva c.c.	Sweat	Dext. Lev.	Flush	Fibril	Notes
1	22	4	142 58 115	0.5	27-21	3-4	155 70 120	2.5	++ 120	Profuse	....	++	+	Chill
2	19	6	75 135	3.0	26-24	1-6	73 195	5.0	++ 260	Profuse	200 D 75 L	++	+	Nausea
3	16	4	75 122	53.0	23-18	2-5	80 133	65.5	++ ++	Profuse	200 D	++	+	.....
4	23	5	70 112	1.5	31-24	2-4	40 130	5.0	124	Profuse	....	++	+	Nausea
5	22	5	90 154	3.0	25-20	4.7	... 150	7.0	++	Profuse	75 L	++	+	.....
6	25	6	120 112	0.0	29-24	4-7	85 130	14.5	++ ++	Profuse	75 L	++	+	.....
7	18	6	40	0.5	28-20	2-6	...	8.5	100 ++	Profuse	....	++	+	.....
8	16	5	... 115	....	21-17	3-5	... 115	3.0	215	Profuse	200 D	++	+	.....
9	18	5	85 110	0.0	17-16	4-5	78 110	2.0	+	Moderate	....	+	—	.....
10	20	5	60	0.0	26-20	4-5	78	1.0	++	Moderate	....	+	—	.....

* Cases 9 and 10 showing negative reactions are tabulated as controls.
† In all cases cold extremities followed the pilocarpin; no sugar.

vertebrae. After bismuth an increasing incoordination of peristalsis ending in faintness and vomiting. Fluoroscope showed on three examinations a fluttering at pylorus. Response to treatment good.

CASE 3.—Diagnosis: angioneurotic edema.

Mrs. B., aged 52, a brass-worker, since 1904 has been subject to localized edema every three or four weeks without fail. It is boggy, firm and does not pit on pressure; it gives a sense of pressure but no pain. In 1907 menstruation ceased. The attacks, which always occurred between the menstrual periods, have continued to date with almost the same regularity. The edema is localized to arms and face, and sometimes spreads to upper part of chest and lasts from four to eight days. Over thirty attacks have occurred in the University Hospital, sixteen being accompanied by convulsions. Urticarial eruptions frequently accompany the swellings.

Fifty-two blood-counts in 1908 showed an average eosinophilia of 60 per cent., while in 1909 there was an average of 59 per cent. They show an increase during an attack with reduction during the intervals.

CASE 4.—Diagnosis: angioneurotic edema.

J. L., schoolgirl, aged 14, last spring, two or three months before menstruation was established, first noticed localized swellings of upper eyelids and at times over brow. The swellings, which are boggy, do not pit on pressure and give no pain, have varied in size, being aggravated by overexertion or mental excitement, and decreased within twelve hours after patient has been in bed. She has had one urticarial wheal on foot. In the past few months she has noticed that her feet and hands are usually cold and feet often wet with sweat; there has been an increasing nasal secretion and marked constipation.

Mother had similar swellings which were especially bad during the menopause, at childbirth and at times when under severe mental anxiety; she has an enlarged thyroid and is of pronounced nervous temperament.

The patient on examination shows slight enlargement of right lobe and isthmus of thyroid, extremities cold, pulse somewhat rapid, waves irregular at times, knee-jerks increased, slight muffling of systolic sound at apex; otherwise no abnormalities detected.

CASE 5.—Diagnosis: fractured coccyx. Enlarged thyroid.

J. K., female, occupation, housework, complains of nervousness and enlarged thyroid. Patient has always had the swelling over the thyroid region unaccompanied by any symptoms until nine months ago, when she had a severe fall, landing on buttocks. Diagnosis of fractured coccyx was then made. Since the fall there have developed general nervousness and mental depression. For three weeks patient has daily periods of unconsciousness lasting from fifteen minutes to one half hour, preceded by chills. Cold and sweating of extremities warned her of attack each time. Patient never utters a cry and no convulsive movements have been noted in any seizure. Attacks are apparently vasomotor in origin. Three months ago patient had frequent attacks of vomiting. Patient is constipated.

Mother has similar swelling in neck.

Examination shows ocular symptoms negative, both tonsils enlarged, adenoids and symmetrical swelling of thyroid which moves with larynx in swallowing; reflexes very prompt; alimentary glycosuria is negative.

Transferred to surgical service, section of coccyx removed and patient discharged free from all symptoms except the thyroid enlargement.

CASE 6.—Diagnosis: Basedow's disease.

Mrs. S. O., aged 44, housewife, complains of swelling of neck, indigestion and palpitation of the heart. She presents the cardinal symptoms of hyperthyroidism, the onset of which followed an attack of rheumatism five years ago. She also complains of nausea, vomiting, eructations of gas and diarrhea.

Slight exophthalmos, lagging of upper lid and widening of palpebral angle are all present. Thyroid is enlarged and firm. Pulsation and palpable venous

thrill are present. Heart: soft systolic murmur at apex and both basal valve areas. Kidneys palpable. No alimentary glycosuria.

Section of thyroid at operation showed epithelial proliferation excessive.

CASE 7.—Diagnosis: bronchial asthma.

W. D., aged 28, drug clerk, eight years ago, following an attack of influenza, developed wheezing respiration, dyspnea and cough. He has had frequent recurrences since then, lasting from twenty-four hours to three months and typical of bronchial asthma. During attacks he is intensely nervous, voids urine frequently and has hot and cold flushes, and epigastric pain. He is greatly helped by epinephrin. Overeating and excitement precipitate attacks. Patient is constipated. He has had nasal catarrh with hypersection and sneezing for ten years; has had polyps and necrotic turbinate bone removed.

Patient given atropin and epinephrin to control attacks.

CASE 8.—Diagnosis: gastric neuroses.

R. C., aged 18, single, farmer, briefly presented the following condition: Gaseous eructations, pulse-rate 60, cold extremities, gastric hyperacidity, constipation.

Examination shows dermographism, large tonsils, cold and mottled extremities, especially hands.

Of the few cases so far examined in which symptoms suggested the possibility of an increased tonus of the sympathetic system, but one gave a positive reaction.

This test, as carried out by Fleischmann, is as follows: The day before the test 100 gm. of dextrose are given on an empty stomach, and the first five hourly specimens of urine are collected, mixed and polarized. The following day another 100 gm. of dextrose are given, and one-half hour later 10 minims of epinephrin (1:1,000) are injected hypodermatically. Just previous to this injection, blood-smears are made for a differential leukocyte count and the average pulse and respiratory rate and blood-pressure estimated. After epinephrin the blood-pressure, pulse and respiratory rates are taken every two minutes, and the patient is observed for tremor and palpitation. At the end of one hour more smears are made for another differential leukocyte count. The urine is collected and polarized as after the first 100 gm. of dextrose. A positive reaction is obtained when there is a marked increase in lymphocytes at the end of one hour, a rise of at least 15 mg. mercury in blood-pressure, a decrease in sugar tolerance and the development of a tremor and sometimes cardiac palpitation.

The patient above mentioned came to the hospital complaining of bilateral paralysis of wrists, a tremulous voice and localized sweating over abdomen. He showed pigmentation of legs and peripheral olive-green staining of cornea, increased reflexes, some tremor and a reduction in sugar tolerance.

The results of his reaction are as follows: Blood-pressure increased from systolic 120 to systolic 140, lymphocytes from 28 per cent. to 43 per cent.; 9.1 gm. of sugar were recovered, as contrasted with a trace before injection of epinephrin. A markedly increased tremor of hands was noted, but no palpitation.

The presentation of the results of the cases, which includes less than half of the series studied, is made with the view of approaching a little nearer to an accurate diagnosis of those diseases so frequently filed away in the records of even the best-regulated hospitals, as neurasthenia, gastric neurosis, etc.

It must be admitted that hypotheses are abundant, and as has been said, I have not been able to support all of them by the analyses of these few cases; certain of them are, however, confirmed, and this newer method of approaching a clear-cut diagnosis is not only of interest, but is worthy, I believe, of still further investigation and development.

Since starting this work my attention has been attracted to the study of a series of cases presented by Barker[6] in 1912, to an article by Neuhof[7] in the same year in which he discusses reflex vagus phenomena, but does not deal with the question of drug reactions, and to Abrams'[8] text-book on spondylotherapy.

In conclusion I wish to extend my thanks to Dr. Stengel for the opportunity of studying the cases, nearly all of which were on his service in the University Hospital, and to Dr. H. B. Wilmer for case 4.

6. Barker and Sladen: Tr. Assn. Amer. Phys., 1912, xxvii, 471.
7. Neuhof: Am. Jour. Med. Sc., May, 1912, cxliii, 724.
8. Abrams: Spondylotherapy, 1912.

EXPERIENCES WITH PROPHYLACTIC TYPHOID VACCINATION

ITS EFFECT ON MENSTRUATION *

ALBERT R. LAMB, M.D.
NEW YORK

Prophylactic typhoid vaccination has won for itself a well-deserved place among the great preventive measures in medicine. This advance has been accompanied by a considerable literature on the history of the procedure, the various methods employed and the results obtained. It is not the object of this article to review the literature. The facts are easily accessible,[1] and, in general, well known.

During the past two years the vaccine has been used prophylactically at the Presbyterian Hospital, New York, and it is the purpose of the present communication in reporting the results of this experience, to emphasize the facts which have seemed most important and to point out in some detail the especial effect of the vaccine on menstruation.

PREVIOUS CONDITIONS

Stimulated by the excellent results obtained by this procedure in the United States Army and by Richardson and Spooner in the training schools of Massachusetts, the medical board of the Presbyterian Hospital, New York, decided, in the spring of 1911, to offer this opportunity of immunization against typhoid to the nurses and attendants at the hospital. Before starting the inoculations, the hospital records were reviewed in order to ascertain the incidence of the disease among those brought into intimate contact with typhoid patients or the cultures of typhoid bacilli. Beginning with the year 1892, the date of the foundation of the training school for nurses, a period of twenty years was covered, during which

* Submitted for publication, Aug. 2, 1913.

* From the Pathological Laboratory, Presbyterian Hospital, Columbia University, New York.

1. The following articles give the main facts of interest and contain references to most of the other important papers:

Russell, F. F.: The Military Surgeon, 1909; Boston Med. and Surg. Jour., 1911, clxiv, 1; Jour. Am. Med. Assn., 1912, lviii, 1331; 1912, lix, 1362; 1913, lx, 344; 1913, lxi, 666; Harvey Lecture, New York, 1913.

Spooner, L. H.: Boston Med. and Surg. Jour., 1910, clxii, 37; Jour. Am. Med. Assn., 1912, lix, 1359.

Davis, D. J.: Jour. Am. Med. Assn., 1912, lviii, 537.

Hachtel and Stoner: Jour. Am. Med. Assn., 1912, lix, 1364.

fifty-two cases of typhoid fever in nurses, doctors, orderlies and bacteriologists were treated in the hospital. Only those cases were included which gave a direct history of contact with typhoid patients or cultures. This gave 2.6 as an average yearly percentage of cases in which typhoid was possibly preventable. There were four deaths, a mortality of 7.69 per cent.

The cases were divided as follows: nurses, 39; orderlies, 6; bacteriologists, 4; doctors, 3.

Of the thirty-nine nurses, nineteen, or about one-half, were in training or on duty in the hospital at the time of contracting the disease. The remaining twenty were caring for typhoid patients when taken ill. There were no deaths among the nurses who developed the disease while in the hospital, but of those who contracted the disease outside, two died. Both were graduates of the hospital. All six orderlies were employed at the hospital in wards in which the care of typhoid patients was necessitated, and there were two deaths. The four bacteriologists developed the disease while working with typhoid cultures. Only one was on duty at the hospital at the time of contracting the disease. The others came from other laboratories. There were no deaths. Of the doctors, two were interns at the hospital and one was a substitute. There were no deaths.

The occurrence of the fifty-two cases by years was as follows:

1892	1	1902	1
1893	0	1903	3
1894	0	1904	4
1895	1	1905	5
1896	2	1906	3
1897	0	1907	6
1898*	11	1908	1
1899	1	1909	0
1900	4	1910	3
1901	4	1911	2

* Spanish War.

It will be seen that in four separate years, 1893, 1894, 1897 and 1909, there were no cases, and that for one period of two years there was also a clean record, facts which must be borne in mind in estimating the value of the results obtained through the use of the vaccine over a comparatively short period of time. In 1898, the year of the Spanish War, there were eleven cases. At that time additional wards were opened for the care of typhoid patients and the hospital staff was greatly overworked. Several of the nurses treated at this time came from the army camps.

The conditions described do not differ essentially from those reported by observers in other localities. Thus Spooner,[2] at the Massachusetts General Hospital in Boston, found that there were three or four cases of

2. Spooner: Am. Jour. Pub. Hyg., 1909, xix, 616.

typhoid a year among the nurses and those especially exposed. He found, moreover, that in these patients the disease ran a course of more than usual severity with a greater number of complications and a higher mortality than the average. I did not find this true in our statistics. The mortality was less than that for the hospital in general and the cases were not unusually severe, nor did they show more complications than the average.

Davis[3] gives no definite figures for St. Luke's Hospital, Chicago, but states that "an occasional nurse has had typhoid."

Joslin and Overlander[4] investigated the conditions in six hospitals in Massachusetts for the years 1902 to 1906 and came to the conclusion that one nurse came down with typhoid for each 114 cases treated, and that "the hospital nurse in Massachusetts is about eight times as liable to contract typhoid fever as the ordinary citizen."

Hachtel and Stoner[5] give higher figures for Baltimore. They find that the nurses and attendants are from twelve to twenty times more liable to contract the disease than the ordinary citizen of Baltimore.

One does not need to cite further specific instances. While differing somewhat in the actual figures, all observers are agreed on the main fact, that nurses, doctors and hospital attendants run a considerably greater risk of contracting typhoid than the ordinary citizen, and that in spite of due precautions, a certain number of them contract the disease each year, the source of infection being the typhoid patient or articles which have come in contact with typhoid cases.

VACCINE

The vaccine used is prepared from an old culture of attenuated virulence, isolated about twelve years ago. The organisms are grown on large agar-slants (agar slanted in 250 c.c. Erlenmeyer flasks is very convenient) for twenty-four hours, washed off with sterile salt solution and heated at a temperature of 53 C. (127.4 F.) for one hour. The vaccine is standardized with the ordinary blood-counting apparatus. After sterility is assured, the vaccine is made up to the required dilution with sterile salt solution containing 0.25 per cent. phenol (carbolic acid). It is put up in small ampules, each containing enough for one dose, and in 30 c.c. vaccine bottles for use where larger numbers are to be vaccinated.

The vaccine was carefully compared with that used in the United States Army, a supply of which was very kindly sent to the hospital by Major Russell. In a large number of inoculations no difference could

3. Davis: Jour. Am. Med. Assn., 1912, lviii, 537.
4. Joslin and Overlander: Boston Med. and Sudg. Jour., 1907, clvii, 247.
5. Hachtel and Stoner: Jour. Am. Med. Assn., 1912, lix, 1367.

be detected in the local or general reactions or in the Widal reactions. Therefore, it would appear that the hospital vaccine represents approximately the same potency and lack of toxicity as that used in the army. The vaccine should be fresh and in general should not be used after it is 3 months old, as it has been shown that deterioration begins at about this time.

DOSAGE

The usual dosage recommended is that employed in the United States Army. It consists of three inoculations at ten-day intervals, the first of 500 million, the second and third of one billion each. This method has been well tried and it has been conclusively shown that it confers a sufficient immunity for a period of at least three years. Conditions there, however, differ somewhat from those among nurses and in private practice where a reaction which would not trouble a man in the regular army is considered quite an event. The same dosage as previously noted was given in a considerable number of the cases in this series, and there were enough troublesome reactions to make one consider a variation of the dose. Of course, one's aim in giving the vaccine is to protect the patient against typhoid and not to avoid reactions; but, if one can produce the desired immunity with the smallest amount of discomfort, the cause of typhoid prophylaxis has been aided just so much. The aim, then, should be to inject the maximum number of bacilli with the minimum amount of discomfort and inconvenience to the patient.

One class of nurses received the four doses, recommended by Spooner,[6] of 100 million, 200 million, 400 million and 600 million at five-day intervals. These cases had fewer and less troublesome reactions than the others, but they received only about one-half the total number of bacilli given by the army method. By considering certain general factors to be mentioned presently, one can considerably increase this total without a corresponding increase in the reactions. It is generally quite safe to make the initial dose 200 million and then, being governed by the reactions which occur, one can very often make the second dose 300 to 400 million, the third 500 to 600 million and the fourth 800 million to one billion. In this way one can more nearly approximate the army total dosage without as many disagreeable reactions.

Some people prefer to take their chances on the possibility of having a reaction rather than submit to the inconvenience of a fourth injection, and in vaccinating large numbers, the extra inoculation means considerable added expenditure of time, especially among women in whom the avoidance of the menstrual period causes many delays and much straggling out of the inoculations. Therefore, it has been the more recent routine to use three graduated doses at from seven-day to ten-day

6. Spooner: Jour. Am. Med. Assn., 1912, lix, 1359.

intervals, the first of 300 million, the second of 700 million and the third of one billion. Here, as in the preceding case, one can be governed by the patient's general condition and can often without fear of undue disturbance, make the first dose 350 to 400 million and the second 800 million.

Of course, it is impossible to predict accurately what reaction a person will have, but one can be governed by certain general facts. In the first place, women are more apt to have reactions than men. This fact is brought out in the statistics of Hachtel and Stoner,[7] and was shown in the first twenty-three cases of this series, of whom twelve were doctors and eleven were nurses. The mild and moderate reactions were about twice as frequent among the latter as among the former. Members of families in which there is, apparently, a marked susceptibility to typhoid or in which the disease has run a severe course, are apt to show more marked reactions. In one family which I vaccinated, both of these facts were true, and of the five members, all showed varying degrees of reaction after all of the inoculations except a boy, aged 11. In one there was the most severe local reaction which I have seen, combined with a moderately severe general reaction and in another, the general reaction after the first two doses was sufficient to discourage a third inoculation. Persons who have a low resistance to any form of infection are apt to have troublesome reactions and the same is true of those who are run down or debilitated from any cause. Those suffering from any chronic disease, should, if vaccinated at all, receive the four smaller doses. Naturally, the preceding statements are only general principles of guidance. One will encounter perfectly robust individuals who come in none of the foregoing classes, who, nevertheless, show severe reactions, while, on the other hand, any of the types mentioned may show no reaction whatever. From my experience up to the present time, I believe that it is best to give to the persons of the types mentioned, the four doses at seven-day intervals, beginning with 200 million and increasing each succeeding dose as far as feasible. For all others, I prefer the three graduated doses, using the upper limits for each dose if possible. From a rather limited experience with children I have seen no reason for graduating the dosage according to body weight. As a rule, they stand the inoculations with less inconvenience than adults and I have seen no troublesome reactions following full-sized doses.

With many people, especially business men, a seven-day interval is preferable to one of ten days. The inoculations can thus be given on successive Saturdays, allowing them until Monday to recuperate from any ill effects. It is best where possible to give the injections in the

7. Hachtel and Stoner: Jour. Am. Med. Assn., 1912, lix, 1367.

late afternoon or early evening so that the maximum reaction may take place while the patients are asleep.

PRECAUTIONS

From my experience it has been apparent that whatever the site chosen for inoculation the patient is inclined to wish that the vaccine had been injected elsewhere. It has been found more convenient and perfectly satisfactory to make the inoculation in the left upper arm, just above the insertion of the deltoid, using the right arm in left-handed people. The vaccine is injected into the subcutaneous tissue, care being taken to avoid entering a vein, as in such cases the general reaction is usually more rapid in its appearance and more severe, and the local reaction more troublesome. In cleaning the arm, iodin and alcohol have been found perfectly satisfactory. After withdrawing the needle, the site of inoculation is touched with iodin and no dressing of any kind is applied, as it has been found that collodion, bandages or gauze with adhesive strips simply add to the local annoyance. In exceptional cases, if the local reaction is moderately severe, a dressing of aluminum acetate has been used with good results.

In prophylactic work, one must remember that patients are not inclined to be particularly grateful for any reactions which the vaccine may cause and it is only human nature for them to attribute to the vaccine every ill for months following. As the value of typhoid vaccine is so great and the need for its universal adoption so striking, it is of the utmost importance to observe every precaution to prevent its falling into disrepute in any case. A careful history should be taken in each case and a sufficiently exact examination made to be sure that the patient is in sound health, for it is quite within the range of possibilities that a person might be inoculated in the early stages of some disease, and it would then be difficult to convince the patient or his friends that the disease was not directly caused by the vaccine. In the present work such incidents were encountered. One nurse developed a typical case of scarlet fever two days after the second inoculation, and for the first day or two even the doctor who saw her considered that she was suffering from a rather severe vaccine reaction with an associated rash. From the subsequent course of the case, there was no doubt as to the nature of the disease. In such an instance in private practice, it would be exceedingly difficult to convince the family that there was no association between the vaccine and the scarlet fever. In another case, a man had an attack of bronchopneumonia which began ten days after the third injection. At the onset he believed that his illness was in some way associated with the vaccine, though in this case the disease followed a very definite exposure to cold. Other cases of association of various diseases with the vaccine will be given later.

It is well recognized that the vaccine may cause a temporary change for the worse in an existing chronic condition. For example, Spooner[8] has encountered temporary and not serious exacerbations in chronic arthritis, chronic cholecystitis, subacute urethritis, furunculosis and acne. I have seen similar instances and will mention them later. Other observers have noted the same thing. While the exacerbations are not apt to be serious, their possibility should be recognized and all chronic conditions should be asked for in the history, as in each case one can then decide whether or not it is wise to give the vaccine. I do not believe that it should be given in cases in which there is a history of tuberculosis; but if, in spite of the existence of some chronic condition, it is decided to go ahead with the vaccine, the patient should be fully acquainted with the possibilities and the vaccination procedure should be followed cautiously and the four smaller doses administered.

Another question which arises is that of vaccination where there is a history of a preceding attack of typhoid fever. It has been found that the reactions in such cases are apt to be rather strikingly severe. In the only case of the kind in which I have used vaccination, that of a young man, there was a moderately severe reaction for a day and a half after the first dose, followed immediately by a severe attack of tonsillitis and in ten days by a profuse urticarial rash. As the disease itself confers a life immunity in almost all cases, and as vaccination in such cases is apt to be followed by bad reactions, I have since refused to inoculate patients who give a history of a previous attack of typhoid. At times this preceding history is not definite and in such cases one must exercise one's own judgment. Where there is a reasonable doubt, I believe one should proceed with the vaccination, using the four smaller doses rather cautiously.

In addition to the above precautions, one should explain the effects of the vaccination to patients and tell them just what they may expect in the way of local and general reactions. Patients should be warned to remain quietly at home while the reaction is present. The most severe reaction seen in the present cases seemed to have been increased by the patient's going about as usual on the day following the first inoculation, when the temperature was 101. The next day the temperature rose to 102.6, and in connection there was a splitting headache and slight epistaxis, while on the following day the temperature reached 100, with the accompaniment of nausea and rather severe diarrhea. Patients should also be warned against taking alcohol in any form until all signs of reaction have disappeared, for not infrequently alcohol has apparanetly added considerably to the severity of the reaction. In this connection, a curious fact was noted in three of these cases. Two patients on the

8. Spooner: Jour. Am. Med. Assn., 1912, lix, 1360.

night following inoculation and one on the second night after, when there was no general reaction and their arms had ceased troubling them, took cocktails just before dinner. Almost immediately afterward their arms, at the site of the inoculation, began to pain so severely that they could not remain at the table.

RESULTS

The inoculations were started in May, 1911. The course of procedure was carefully explained and eleven nurses and twelve doctors volunteered for the preliminary vaccinations. In these twenty-three cases, only two inoculations were given, the first of 400 million and the second, after an interval of ten days, of 650 million. In seventeen of these cases, microscopic Widals were done ten days after the first injections, the Widals prior to the vaccination having been negative in all cases at 1:20. In five the Widal was negative in a dilution of 1:20, in twelve it was positive and in two it was positive at 1:100. The results of the Widal test following the second inoculation may be seen in Table 1.

TABLE 1.—RESULTS OF WIDAL TEST IN TWENTY-TWO CASES*

Dilution	Reaction	Number	Percentage
1-20	Negative	3	13.63
1-20	Positive	19	86.36
1-100	Positive	12	54.54
1-200	Positive	6	27.27
1-400	Positive	6	27.27
1-1600	Positive	1	4.54

* Tests made ten to fourteen days after second inoculation.

After these preliminary inoculations, the work was taken up as a routine with each succeeding class in the training-school (Table 2). During the first few months the members of a new class are not on ward duty, and as some leave the school and others enter, the vaccinations are not started until the nurses have been here for several months. Then the class is called together, the vaccination and its importance explained and the vaccine offered to those who desire it. Vaccination is still entirely voluntary in the training-school.

The following table shows the percentage of each class vaccinated:

TABLE 2.—PERCENTAGE OF VACCINATIONS OF NURSES

Class of	In class	Number eligible	Vaccinated	Percentage
1911	29	26	16	61.5
1912	33	31	22	70.9
1913	38	32	27	84.3
1914	13	11	11	100.
1915	30	25	23	92.
Totals	143	125	99	79.2

This table shows that as the work became better known and appreciated, a larger number of the nurses wished the vaccination. Thus in the last two classes, 94.4 per cent. of those eligible for the vaccination have taken it.

Since May, 1911, 143 nurses have been in training. Of these, 99 were vaccinated, 26 refused; in 3 it was considered inadvisable on account of some form of chronic illness, 14 had recovered from typhoid and one had been inoculated with the vaccine before entering the school. Thus, 79.2 per cent. of the eligible nurses were vaccinated and 20.8 per cent. remained unprotected, including the three who wished the vaccine, but whose health did not warrant its use.

In addition, during the same period, May, 1911, to June, 1913, 33 doctors and medical students connected with the hospital, 7 employees in the pathologic department, where the vaccine is compulsory, and 64 outside cases have been vaccinated, making a total of 203 cases; and in this period there have been no cases of typhoid fever either in the training-school or among those vaccinated. There have been no untoward effects from the vaccine and no instances of arm infection.

LOCAL AND GENERAL REACTIONS

Local and General.—The local reactions were similar in all respects to those usually observed.

The general reactions are shown in Table 3. In classifying these reactions, the temperature is taken as the general index as in army cases, but some reactions are put down as mild or moderate where the constitutional disturbances were sufficient to warrant it even though the temperature remained low. It was noted that some of the nurses showed subnormal temperatures following the inoculations, which was often associated with such constitutional disturbances as dizziness, nausea and vomiting.

TABLE 3.—PERCENTAGE OF GENERAL REACTIONS

Reaction	First Dose 203 Cases		Second Dose 200 Cases		Third Dose 169 Cases		Fourth Dose 27 Cases	
	No.	Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.
None.....	96	47.6	111	55.5	140	82.8	24	88.8
Mild.....	88	43.3	71	35.5	19	11.2	3	11.1
Moderate..	18	8.9	18	9	11	6.5	0	....
Severe.....	1	0.49	0	....	0	....	0	....

It will be noted that of these cases, 184, or 90.6 per cent., showed either no reaction or a mild one after the first dose; 182, or 91 per cent., after the second; 159, or 94.1 per cent., after the third, and 27, or 100 per cent., after the fourth. The symptoms complained of in the order of frequency were: malaise, headache, general aching, nausea, dizziness, anorexia, vomiting, diarrhea, epistaxis, faintness.

One hundred and ninety reported at the end of six months that they were perfectly well, three had suffered from fatigue for two or three

months, but had recovered, and two thought that their health was still impaired by the vaccine.

WIDAL REACTIONS

The results of the microscopic Widal in fifteen consecutive cases tested ten days after third inoculation, may be seen in Table 4.

TABLE 4.—RESULTS OF WIDAL TEST IN FIFTEEN CASES*

	Positive Reactions	
Dilution	No.	Per cent.
1-20	15	100
1-100	15	100
1-200	11	73.3
1-400	9	60
1-800	5	33.3
1-1600	3	20
1-2000	2	13.3
1-12000	1	6.6

* Test made ten days after third inoculation.

The Widal reaction has been so definitely worked out by so many observers that it is now used here only in exceptional cases and to control new vaccine.

MENSTRUATION

After the first few inoculations the very definite impression was gained that menstruation was apt to be affected by the vaccine. Consequently, careful records of this particular phase of the work were kept in order to determine the amount of the disturbance. A comparison of the army vaccine with that used in the hospital showed no difference in their liability to cause menstrual irregularities. Other workers have briefly noted that these irregularities occur, so that one is justified in concluding that the effects are not due to the peculiarities of any one particular vaccine. A correct judgment of the part played by typhoid vaccine in menstrual disturbances is difficult and any statistics are open to several sources of error.

In the first place, it is well known that nurses are apt to have menstrual irregularities during the first few months of training, which are due to change of environment and method of living. In this work, the vaccinations were not started until the nurses had been in the hospital for from three to six months, and these nurses did not show any more frequent or more marked changes than did the nurses who had been in training for from one to three years. Again, some women are always more or less irregular. Where there was such a history, any disturbances during the course of the inoculations, unless of striking character, were not considered, and the case was put down as unaffected. In some, temporary causes other than the vaccine may have been the causal factors. It is difficult to eliminate such causes and they introduce a certain unavoidable source of error.

In compiling the records of the one hundred cases in which menstruation was studied, only those cases are put down as affected in which the evidence of disturbance by the vaccine seemed to me quite definite, after ruling out all other apparent causes and where the patients themselves felt that the condition was distinctly unusual for them.

Perhaps one can gather the best idea of the nature of the disturbances and their close association with the vaccine by a brief description of some of the more striking cases.

REPORT OF CASES

Case 1.—Vaccination finished early in June. The two following periods were missed. In August appendix was removed and menstruation did not occur again until December. It has been normal since then.

Case 2.—For three periods following the vaccination was about three weeks late each time. Then normal.

Case 3.—Missed period following vaccination and then a condition of scanty flow a week or more late existed for three months.

Case 4.—Menstruated every two to three weeks for three months following the vaccination.

Case 5.—After the third inoculation came on two weeks ahead of time and caused more discomfort than ever before.

Case 6.—Vaccinated the day after menses had ceased. Menses returned again that night.

Case 7.—During the course of vaccination menses came on one week ahead of time, causing patient to go to bed.

Case 8.—Twelve days early. More profuse and of longer duration.

Case 9.—After first inoculation menses ten days early, more painful and scantier.

Case 10.—After third inoculation menses ten days early, more painful and scantier.

Case 11.—Never quite regular. Much worse after vaccination. Skipped two months entirely and later menstruation was more painful.

Case 12.—Five days late after the second inoculation. One week early after the third.

Case 13.—Skipped two periods. Next time in three weeks. Then in less than three weeks.

Case 14.—Skipped three periods.

Thus in fourteen cases there were very distinct changes, due, in all probability, to the effect of the vaccine itself.

A complete tabulation of all the cases shows that a little more than one-half, 53 per cent., showed some type of menstrual disturbance, distinct though at times quite trivial, while 47 per cent. were unaffected. The types of disturbance may be seen in the following table, more than one type occasionally appearing in the same individual:

Menstruation early for one or more periods	25
Menstruation more painful for one or more periods	18
Menstruation late for one or more periods	15
Menstruation more profuse for one or more periods	10
Menstruation skipped for one or more periods	7
Menstruation scantier for one or more periods	6

It should also be noted that where these irregularities occur the patients are more apt to have associated with them a more troublesome general reaction.

The number of cases which show some change is rather striking, even allowing for unavoidable errors. If one merely considers the fourteen very definite instances cited, the percentage is high enough to show that a very striking relationship exists between the vaccine and menstrual disturbances, a fact which is of interest in view of the similar disturbances seen in typhoid fever. All of the cases included above were followed for at least six months. In none was there any disturbance at the end of this time. Therefore, it would appear that the changes are of only temporary significance and that they carry with them no lasting ill effects.

In this connection, one must consider the possible consequences of vaccination during pregnancy. I have a record of only one such case. In this instance, the woman's husband was ill with typhoid and much of his care fell on her. Consequently, in spite of her pregnancy, vaccination was carried out. She did not develop typhoid, nor did she have any ill effects from the vaccine. The menstrual disturbances have impressed me sufficiently, however, to make me conservative about vaccinating pregnant women. At the present time, I would not vaccinate under such circumstances, except under some special indication, such as that shown in the preceding case.

Recognizing that menstrual disturbances do occur, one naturally turns to the question of reducing them to a minimum. I believe that, barring some special indication for haste, the vaccination of women should be so arranged that the first inoculation will come a few days after a period. The second and third injections can then be given at seven-day intervals, leaving about a week's leeway before the succeeding period. If, for any reason, ten-day intervals are necessary, it is best to postpone the third inoculation until a few days after the second period. By giving the vaccine in this way, menstrual difficulties are reduced, though not entirely eliminated.

ASSOCIATION WITH OTHER DISEASES

It has already been mentioned that numerous instances of aggravation of existing chronic conditions are on record. It is, therefore, of interest in a series of cases like the present, where most of the cases can be followed, to note the relation of the vaccine to the development of various diseases.

Diseases of the Respiratory Tract.—Thirteen patients developed symptoms of some such affection during the course of the vaccinations, or so soon afterwards that they attributed them to the vaccine. Five patients developed coryza beginning within one or two days after an

injection, and in one case the cold lasted for six weeks. There were five cases in which tonsillitis began within one or two days after an inoculation. In one case bronchitis developed during the course of the vaccination, and the disease lasted for two months. One patient, already referred to, contracted bronchopneumonia ten days after the third injection. In this case there was definite exposure to cold.

Probably many of these cases were merely coincidences, though one receives the very definite impression that in some cases, at least, the patient's resistance is sufficiently lowered to favor the chances of such infections.

Appendicitis.—This condition was noted in five cases. In only one, however, was the association sufficiently close to justify suspicion of the vaccine as an accessory cause. In this case, there were moderately severe reactions associated with nausea, vomiting and diarrhea, after all three doses. Very soon after the third dose the patient began to have pain in the region of the appendix, which in the course of a short time necessitated an operation.

Tuberculosis.—Three patients in the present series have developed pulmonary tuberculosis since receiving the vaccine. In no case was there any previous history of the disease, and in none was the relation between the vaccine and the disease sufficiently close to be suggestive.

Tachycardia.—In one case there was a history of mild attacks of tachycardia. The patient had two such attacks on the eleventh and twelfth days following the second inoculation, and a third attack within five hours after the third injection. In another case, that of a man who normally had a very slow pulse, the rate doubled within a few hours after receiving the first dose. In a third case with a history of attacks of rapid heart action and arhythmia, there was no effect from the vaccine.

Furuncles.—In one instance a patient began to suffer from furuncles about two weeks after the third injection.

Scarlet Fever.—The development of a case of scarlet fever during the course of the injections has already been mentioned.

Scabies.—One patient was suffering from a rather extensive case of scabies, when he presented himself for the vaccination. He was warned that the vaccine might cause an aggravation of the skin condition, but he desired the vaccine in spite of this possibility. Much to our surprise, without any treatment for the skin affection, it almost completely disappeared during the course of the inoculations, but reappeared a little later.

HEXAMETHYLENAMIN: THE LIBERATION OF FORMALDEHYD AND THE ANTISEPTIC EFFICIENCY UNDER DIFFERENT CHEMICAL AND BIOLOGICAL CONDITIONS *

PAUL J. HANZLIK, M.D., AND R. J. COLLINS, A.B.
CLEVELAND

Ever since the recognition of hexamethylenamin as a beneficial therapeutic agent in vesical conditions by Nicolaier[1] in 1894, the use of this drug has steadily increased. It has been recommended as an antiseptic agent for practically all of the body fluids, largely on the basis of the fact that after internal administration it has been found present everywhere in the body. Although it has been quite generally recognized that hexamethylenamin is practically non-toxic, the criteria for its action as an antiseptic have only comparatively recently begun to be appreciated. Its decomposition into ammonia and formaldehyd is well known, and it is generally believed by pharmacologists that the bactericidal properties of hexamethylenamin are due to the liberated formaldehyd. However, there has been no absolute proof that free hexamethylenamin may not prevent bacterial growth. Indeed, it is maintained by some clinicians that hexamethylenamin is bactericidal and that the presence of free formaldehyd is not necessary when it is to be used as an antiseptic.

The conditions under which formaldehyd may be liberated from hexamethylenamin have been ascertained largely from experiments *in vitro*. Some of the factors are changes in temperature; time allowed for the decomposition to take place, and reaction of the solution. The last is, undoubtedly, the most important factor. In general, acids are known to facilitate its decomposition, while alkalies prevent it. There has been no thorough study of the influence of these factors under conditions simulating those of the body, and especially in the living organism. This is especially necessary in the light of the modern conceptions of the true reactions of the various body fluids, according to Sörensen, L. J. Henderson and others. Tests for the appearance of hexamethylenamin and formaldehyd in urine and other body fluids have been quite

---

* From the Pharmacological Laboratory, Medical School of Western Reserve University.

* Submitted for publication August 15, 1913.

* The expenses of this research were defrayed in part by a grant from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association.

1. Nicolaier, A.: Deutsch. med. Wchnschr., 1895, No. 34, p. 541; Centralbl. f. d. med. Wissensch., 1894, xxxii, 897.

extensively employed, but often with confusion as to their delicacy and clinical applicability, and without due appreciation of their worth and significance. Tests have been accepted as conclusive for formaldehyd which do not differentiate between free hexamethylenamin and free formaldehyd; certain tests are thought to be best suited for clinical application, others for pure chemical work only. Therefore, we have attempted to gain more definite information concerning the applicability of various tests for free formaldehyd and free hexamethylenamin; to study the various conditions under which the liberation of formaldehyd takes place, and to settle definitely whether hexamethylenamin itself is bactericidal or not.

In this work we have sought to correlate as much as possible the chemical data obtained with experiments *in vitro* and on the lower animals with observations on patients. The clinical material was obtained from the medical wards and dispensary clinics of Lakeside Hospital and Western Reserve University. For a description of the methods used, the sections to follow must be consulted.

The more important results of our work may be briefly stated as follows: The phloroglucin test is the most delicate and most useful test for free formaldehyd. Free alkali prevents the liberation of formaldehyd from hexamethylenamin. Liberation of formaldehyd from hexamethylenamin depends on the hydrogen ion concentration of the solution; that is, it depends on true acidity. After the administration of hexamethylenamin, free formaldehyd does not occur in blood, cerebrospinal fluid and all other body fluids which are truly alkaline (concentrations of hydrogen ions less than 7.0), nor does it occur in alkaline urine; but it always occurs in the urine of animals and of man when the reaction is acid, that is, when the hydrogen ion concentration is greater than 7.0 (neutrality). Definite and direct proof is offered that hexamethylenamin itself is not bactericidal.

I. Tests for Formaldehyd and Hexamethylenamin

Bromin-Water Test for Free Hexamethylenamin.—This test is described by Nicolaier[2] as follows: Fresh bromin-water gives an orange-yellow precipitate when added directly to solutions containing hexamethylenamin. The precipitate forms at the moment of contact with bromin water, but redissolves until three to four drops of the reagent have been added. The precipitate forms when the merest trace of hexamethylenamin is present. No precipitate is formed with free formaldehyd. The test is applicable to urine, but to no other body fluids containing proteins such as blood, blood-serum, cerebrospinal fluid, pleural, pericardial and synovial fluids and urine containing albumin, because the proteins themselves form a precipitate. In order to test for hexamethylenamin in fluids in which the bromin-water test is not applicable, the fluid is distilled alone or with the addition of a little mineral acid and either of the following tests for formaldehyd are applied to the distillate.

2. Nicolaier: Ztschr. f. klin. Med., 1899, xxxviii, 350.

Tests for Free Formaldehyd.—Many of the most widely employed tests for free formaldehyd involve the use of acids, which would decompose the hexamethylenamin, and would therefore be unsuited for distinguishing between free formaldehyd and hexamethylenamin. Only those which are made in weakly alkaline reaction are suitable. This limits the choice practically to the phenylhydrazin and phloroglucin tests.

Phenylhydrazin-Nitroprussid Test for Free Formaldehyd.—This test was first described by Rimini,[3] and later by Arnold and Mentzel.[4] Three reagents are necessary: (1) Phenylhydrazin hydrochlorid 0.5 per cent. in water; (2) sodium nitroprussid[5] 5 per cent. in water; (3) sodium hydroxid 10 to 20 per cent. The test is performed in the cold or at ordinary room temperature as follows: To 5 to 10 c.c. of the fluid to be tested contained in a test-tube are added 3 drops of the phenylhydrazin, 2 drops of the nitroprussid and 3 drops of the alkali in the order mentioned. If formaldehyd is present in an aqueous solution, an emerald green to a deep blue color is formed (depending on the concentration of formaldehyd) at the moment the alkali comes in contact with the fluid. This color gradually diffuses throughout the fluid and almost at once begins to disappear, particularly in highly dilute formaldehyd solutions, assuming finally an orange-yellow to a wine-red color. With water alone, the test gives a greenish-yellow at the moment the alkali is added, making it entirely indistinguishable from very dilute solutions of formaldehyd (1:2,000,000 and higher). The change to wine-red occurs much more rapidly in water than in solutions containing formaldehyd which are positive with the test. In urine containing formaldehyd the sequence of colors is as follows: As soon as the hydroxid is added, a deep purple color (generally, but not always) is formed. This quickly changes to green, then to yellow and finally to a yellowish-red. The test is directly applicable to all body fluids except bile and whole blood, owing to the color possessed by these fluids.

Phloroglucin Test for Free Formaldehyd.—This was first described by Jorissen.[6] The reagent used consists of phloroglucin (Reagent-Merck) 0.1 gm. dissolved in 10 c.c. of 10 to 20 per cent. sodium hydroxid. When first prepared the solution acquires a bluish-violet color, but on standing becomes entirely colorless or with at most a yellowish tinge. The reagent may be used freshly prepared since the violet color does not interfere with the red of formaldehyd. The test is performed in the cold or at ordinary room temperature by the direct addition of about 0.5 c.c. of the reagent to about 1 to 2 c.c. of the fluid containing formaldehyd. A deep bright red appears instantaneously with higher concentrations of formaldehyd, but with lower concentrations of formaldehyd it requires about one-half to one minute for the color to reach its maximum intensity. The color persists for at least five minutes with dilute solutions and much longer with concentrated formaldehyd solutions.[7] The test is directly applicable to all body fluids except whole blood and bile. The hemoglobin of fluids containing a trace of blood (enough to give a red tint) is immediately reduced by the alkali of the reagent, the solution assuming a yellowish color, and does not interfere with the formaldehyd reaction. The reagent added to water alone gives a clear colorless solution.

Delicacy of the Formaldehyd Tests.—This was tested out in the following manner: Known dilutions of formaldehyd[7] ranging from 1:1,000 to 1:15,000,000 were made in light amber-colored urine and in water. The tests were then performed in the manner described above. The results obtained (Table 1) were as

3. Rimini: Ann. di. Farmacol., 1897, xvii, 97.

4. Arnold and Mentzel: Ztschr. Nahr. Genussm., 1902, v, 353.

5. In place of the nitroprussid, sodium ferricyanid may be used, in which case the resulting color is red.

6. Jorissen: Bull. soc. chim. Belg., 1897-98; xi; xii, 211.

7. The concentrations of formaldehyd throughout the paper refer to absolute formaldehyd and not to the commercial (40 per cent.) solution.

follows: With the phenylhydrazin, shades of blue to green with the higher concentrations to a greenish-yellow with a concentration of 1:2,000,000, indistinguishable from that of the test plus water. The limit of delicacy obtained was 1:1,000,000. With the phloroglucin test a fine gradation of red colors was obtained, ranging from a deep bright red with the higher concentrations to a visible trace of pink in 1:10,000,000. The results with urine were similar.

RELATIVE USEFULNESS OF THE TWO TESTS

Certain objections have been raised to the use of delicate tests for formaldehyd in clinical practice. Burnam[8] has proposed the use of phenylhydrazin test as a practical clinical test owing to its lesser delicacy,

TABLE 1.—DELICACY OF FORMALDEHYD TESTS*

Concentration of Formaldehyd	Phenylhydrazin Test	Phloroglucin Test
1:1,000	+ Deep blue	+ Very deep red
1:10,000	+ Blue	+ Less deep red
1:50,000	+ Bluish green	+ Lesser deep red
1:100,000	+ Green	+ Deep red
1:250,000	+ Green	+ Lighter deep red
1:500,000	+ Light Green	+ Light red
1:1,000,000	+ Very light green	+ Lighter red
1:2,000,000	+ (?) Very light greenish	+ Very light red
1:4,000,000	+ (?) Very light greenish	+ Light pink
1:8,000,000	+ (?) Very light greenish	+ Very light pink
1:10,000,000	+ (?) Very light greenish	+ Visible trace of pink
1:15,000,000	+ (?) Very light greenish	— Colorless
Distilled water	+ (?) Very light greenish	— Clear; colorless

* In this table the plus sign (+) means that the test was positive; the minus sign (—) means that the test was negative.

thus serving as a reliable index of therapeutically efficient quantities of excreted formaldehyd. We have found this test to be comparatively delicate (1:1,000,000) and capable of detecting concentrations of formaldehyd below the margin of bactericidal efficiency. The phenylhydrazin test requires three reagents, two of which are unstable; and the several changes of color in urine add an element of complexity. On the

8. Burnam: THE ARCHIVES INT. MED., 1912, x, 324.

other hand, the phloroglucin test gives a constant red color with gradations of intensity depending on the concentration of formaldehyd; its application is exceedingly simple; it requires only one reagent, and for these reasons alone should be preferred to the phenylhydrazin as a more practical test. These conclusions have been reached from an extensive application of both tests simultaneously in the greater portion of the experiments embodied in this paper, as well as on other occasions.

CONCLUSION

The phloroglucin test for free formaldehyd (limit 1:10,000,000) is more sensitive than the phenylhydrazin (limit 1:1,000,000); it is simpler and does not give with urine the complexity of colors which is so troublesome with the phenylhydrazin test.

II. Acid Facilitates, Alkali Inhibits the Liberation of Formaldehyd from Hexamethylenamin in Body Fluids

The comparative ease with which hexamethylenamin decomposes in aqueous acid solution and its lack of decomposition in aqueous alkaline solutions might conceivably be somewhat modified in body fluids, for instance, by ferments. In the experiments which follow, various body fluids and water were used, and the effects of reaction, time and temperature were studied.

Different body fluids and water were mixed with hexamethylenamin, 0.1 and 0.5 per cent., the reaction made acid by the addition of 0.2 per cent. hydrochloric acid; or alkaline by 0.2 per cent. sodium carbonate or 0.1 per cent. ammonium hydroxid. The mixture was divided into three sets of duplicate tubes of 5 c.c. each. The phloroglucin and phenylhydrazin tests were applied (1) to one pair of tubes as soon as the dilutions were made; (2) to a second portion after the solutions were raised to the boiling point, and (3) to a third portion after the solutions were incubated for one and five hours at 37.5 C. (99.5 F.). The data obtained have been placed in Table 2. The results with both the formaldehyd tests were identical, and they have been included under one column. The doubtful experiments marked (?) in the table can probably be considered negative in the light of our further experience.

COMMENT ON TABLE 2

Freshly-Made Solutions.—Formaldehyd was liberated at once in all the acidulated fluids except in the cat's serum in which the acid was probably neutralized; this gave the test on standing. In the fluids alone, formaldehyd could be detected only in infected ascitic fluid and the stronger pancreatic extract. The composition of the ascitic fluid was unknown and it may have contained substances giving rise to true acidity

TABLE 2.—Liberation of Formaldehyd from Hexamethylenamin in Various Body Fluids*

Hexamethylenamin 0.5 Per Cent. in	Freshly Made Solutions				Solutions Raised to the Boiling Point				Solutions Incubated for Five Hours at 37.5 C.			
		Fluid Containing										
	Fluid alone	0.2 Pct. HCl	0.2 Pct. Na_2CO_3	0.1 Pct. Ammonium Hydroxid NH_4OH	Fluid alone	Fluid Containing 0.2 Pct. HCl	Fluid with 0.2 Pct. Na_2CO_3	Fluid with 0.1 Pct. NH_4OH	Fluid alone	Fluid with 0.2 Pct. HCl	Fluid with 0.2 Pct. Na_2CO_3	Fluid with 0.1 Pct. NH_4OH
Water	—	+	—	....	+	+	+?	....	+	+	—	....
Sodium chlorid 5 per cent.	—	+	—	....	+	+	+?	....	+	+	—	....
Urine	—	+	—‖	....	+	+	+**	....	+	+	+	....
Extract cat's pancreas (50 per cent. glycerol)	—†	+	—	....	+	+	—**	....	+	+	—	....
Cat's serum	—	—	+¶	....	+	+	+	....	+	+	±	....
Egg albumin sat'd.	—	+	—	....	+	+	—	....	+	+	—	....
Ascitic fluid (sterile)	—	+	—	....	+	+	—	....	+	+	—	....
Ascitic fluid (infected)	+	+	—	....	+	+	—	....	+	+	—	....
Fluid of mesenteric cyst‡	—	+	+?	....	+	±	+?	....	+	+	+?	....
Hexamethylenamin 0.1 per cent. in...	..	..	..	....	..	..	...	....	Solutions Incubated for One Hour at 37.5 C.			
Water	—	+	—	—	+	+	+	—	+	+	+?	—
Sodium chlorid 2 per cent.	—	+	—	—	+	+	±	—	+	+	±	+?
Urine	—	+	—	—	+	+	—	—	+	+	—	—
Egg albumin, sat'd.	—	+	—	—	+	+	—	—	+	+	—	—
Pancreatic extract	+	+	—	—	+	+	—	—	+	+	—	±
Cerebrospinal fluid§	—	+	—	—	+	+	—	—	+	+	—	—

* In the table + = present; — = absent; +? = doubtful or slight; ± = one test positive, the other negative.

¶ Stood fifteen minutes.

‖ Standing over night showed trace of formaldehyd by the phloroglucin test, none by the phenylhydrazin test.

† Standing for two hours showed a trace of formaldehyd by the phloroglucin test.

** On standing over night, then heated, both tests showed the presence of formaldehyd.

‡ Examination of this fluid by Dr. Pilcher showed a thick, creamy emulsion; slightly alkaline to litmus; fat, 25.2 per cent. globulin and albumin present; trace of albumose; trace of dextrose; became rancid on standing.

§ Represents a collection from several individuals with different clinical conditions.

(not litmus acidity). No explanation can be offered for the behavior of the pancreatic extract. Formaldehyd was absent in practically all of the alkaline (Na_2CO_3 and NH_4OH) fluids.

Solutions Raised to the Boiling Point.—The data indicate the uniform presence of formaldehyd in acid fluids as well as in the fluids alone. Boiling liberates formaldehyd from all the fluids except when alkali had been added. The majority of alkaline fluids were negative, but a few appeared positive after boiling. The reactions of these fluids had not been observed, however, and this may explain the discrepancy.

Incubation for Periods of One Hour and Five Hours at 37.5 C.—The results for the different periods of incubation were the same and about identical with those when the solutions were raised to the boiling point. That is, formaldehyd was uniformly present in the fluids alone as well as in the acidified fluids, and absent in all of the alkaline fluids. This in general indicates the ease with which hexamethylenamin decomposes even at a moderate temperature.

CONCLUSION

Solutions of hexamethylenamin in water and the various body fluids used do not liberate formaldehyd immediately, but do so on boiling, or on standing one hour at 37.5 C., or at once if acid is added. Alkalies prevent the liberation, except in a few doubtful instances.

III. Formaldehyd Liberation and Culture Experiments with Hexamethylenamin in Pathological Fluids

The experiments hereafter related were made to determine directly the antiseptic effects of various proportions of hexamethylenamin in body fluids, and to observe whether the antiseptic effects go parallel with liberation of formaldehyd. This direct determination seemed worth while, because the higher protein content, etc., of such fluids might conceivably modify theoretical effects. The culture experiments were made by Dr. H. O. Ruh, Lakeside Hospital, as follows:

With each pathological fluid a series of tubes was prepared, each containing 7 c.c. of fluid, with different amounts of hexamethylenamin or formaldehyd. To each tube was added 1 c.c. of a very dilute suspension of bacteria (pure cultures of the different varieties). The tubes were then shaken and placed in an incubator at 37.5 C. At the end of twenty-four and forty-eight hours the character of the bacterial growth was determined by direct inspection. Turbidity indicated bacterial growth; a clear fluid indicated no growth. Some of the tubes showed differing grades of turbidity, but as the cultures were not constant in this respect no differentiation was attempted between them. In some cases the original fluids were so turbid that plate cultures were made. Here the presence of colonies indicated bacterial growth; no colonies meant no growth. The phenylhydrazin and phloroglucin tests were applied to each tube at the end of the incubation period. The results of our experiments have been placed in Table 3. The results of the formaldehyd tests were identical and have been placed in a single column.

TABLE 3.—FORMALDEHYD LIBERATION AND CULTURE EXPERIMENTS WITH HEXAMETHYLENAMIN IN PATHOLOGICAL FLUIDS*

TABLE 3.—Formaldehyd Liberation and Culture Experiments with Hexamethylenamin in Pathological Fluids*

Hexamethylenamin	Fluid and Micro-Organism																	
	Bouillon (alone)		Pleural Transudate† with M. Staphylococcus		Pleural Transudate‡ with B. Typhosus		Ascitic Fluid§ With B. Typhosus		Hydrocoele Fluid** with M. Staphylococcus		Ascitic Fluid¶ with B. Pyocyaneus		Ascitic Fluid (sterile) with B. Typhosus		Chest Fluid (alone)		Bouillon with M. Staphylococcus	
	B	F	B	F	B	F	B	F	B	F	B	F	B	F	B	F	B	F
1:200	....	....	....	....	....	....	....	....	+	+	+	—	+	+	..	..	..	..
1:300	....	....	....	....	....	....	....	....	+	+	+	—	+	+	..	..	..	..
1:500	....	—	+	+	+	+	+	+	+	+	+	—	+	+	..	..	+	..
1:1,000	....	—	+	+	+	+?	+	+	+	+	+	—	+	+	..	..	+	..
1:2,000	....	—	....	....	....	....	....	....	+	+	+	—	+	+	..	..	+	..
1:5,000	....	—	+	+	+	+?	+	+	..	..	..	..	+	+	..	..	+	..
1:10,000	....	—	+	—	+	—	+	+	..	..	..	..	..	..	..	..	+	..
1:50,000	....	—	+	—	+	—	+	—	..	..	..	..	..	..	..	..	+	..
Formaldehyd	Bouillon with B. Staphylococcus																	
1:1,000	—	+	—	+	—	+	—	+	—	+	—	+	+	+	..	+	—	+
1:2,000	—	+	—	+	—	+	—	+	..	..	—	+	+	+	..	+	—	+
1:5,000	—	+	—	+	—	+	—	+	—	+	—	+	+	+	..	+	—	+
1:10,000	—	—	—	+	+	+	—	+	..	..	+	+	+	+	..	+	—	+
1:20,000	+	—	+	+	+	+	—	+	+	+	+	—	+	+	..	+	+	—
1:40,000	+	—	+	+	+	+	—	+	+	+	+	—	+	+	..	+	+	—
1:80,000	+	—	+	—	+	+	—	+	..	..	+	—	+	—	..	+	+	—

* B = bacteria; F = formaldehyd; + = present; — = absent.

† Pleural transudate from a patient with valvular incompetency and synechiae cordis. The fluid was slightly cloudy and contained a few erythrocytes, therefore plate cultures were made.

‡ Pleural transudate from a patient with valvular disease. The fluid was very slightly turbid.

§ Ascitic fluid from a case of hepatic cirrhosis. The fluid was very slightly turbid. Slight increase in cloudiness produced by bacterial growth could easily be made out.

** The fluid was clear, colorless, and sterile.

¶ Ascitic fluid from a case of hepatic cirrhosis. The fluid was slightly turbid and contained a few erythrocytes. Plate cultures were made.

Ten per cent. dilutions of sterile bile in 0.9 per cent. sodium chlorid were incubated with *B. typhosus*, *M. staphylococcus aureus*, and *B. pyocyaneus* in tubes as well as in plates, but the results were unsatisfactory on account of the darkness and turbidity of the media.

TABLE 5.—Bactericidal Proper

Micro-Organism	Culture Medium	Character of Bacterial Growth and Presence		
		1:50	1:100	1:200
M. pyogenes aureus	Agar alone	None (1), (2) +	×	.....
M. ureae	Agar alone	None (1) +	×	.....
B. diphtheriae	Agar alone	no colonies (1) + strong	No colonies (1) + strong	.....
B. typhosus	0.2 per cent. CO_3-agar	.....	Diffuse (1) (2) —	×
B. typhosus	0.2 per cent. CO_3-agar	None (1) 10 colonies (2) +	None (1) Diffuse (2) +?	1 colony (1) 6 colonies (2) —
B. typhosus	0.5 per cent. NH_3-agar	None (1), (2) + trace	None (1), (2) —	×
B. typhosus	0.5 per cent. CO_3-agar	None (1) several small colonies (2) —	None (1) Several colonies (2) —	×
M. pyogenes aureus	0.5 per cent. CO_3-agar	diffuse (1) (2) —	×	×
M. pyogenes aureus	0.5 per cent. NH_3-agar	None (1) diffuse (2) +	None (1) Diffuse (2) —	×
M. pyogenes aureus	0.5 per cent. NH_3-agar	diffuse (1) +	Diffuse (1) —	×
M. pyogenes aureus	0.5 per cent. NH_3-agar	Numerous colonies (1), (2) +?	Numerous colonies (1), (2) —	.....
B. coli	0.5 per cent. NH_3-agar	Many colonies (1) + slight	Many colonies (1) —	.....
B. pyocyaneus	0.5 per cent. NH_3-agar	Very marked growth (1) +?	Very marked growth (1) —	.....
B. coli	0.5 per cent. NH_3-agar	None (1) + trace	Marked growth (1) —	.....
Actinomycosis	0.5 per cent. NH_3-agar	12 colonies (1) + trace	12 colonies (1) —	.....
M. ureae	0.5 per cent. NH_3-agar	Numerous colonies (1) +?	Numerous colonies (1) —	.....
B. pneumoniae	0.5 per cent. NH_3-agar	Numerous colonies (1) +	Numerous colonies (1) —	.....
B. anthrax	0.5 per cent. NH_3-agar	Fewer colonies than rest (1) +	Great many colonies (1) —	.....
B. diphtheriae	0.5 per cent. NH_3-agar	Diffuse cloudiness (1) (2) +?	Diffuse colonies (1) (2) —	.....
B. diphtheriae	0.5 per cent. NH_3-agar	diffuse (1) +	Diffuse (1) —	.....

* CO_3-agar refers to agar modified with sodium carbonate; NH_3-agar refers "×" are the same as the next higher concentration.

(1), growth at end of twenty-four hours; (2), at end of forty-eight hours;

of Formaldehyd in Hexamethylenamin			Remarks
1:250	1:500	Blank	
Numerous colonies (1), (2) + ×	×	Numerous colonies (1), (2) — Numerous colonies (1)	
88 colonies (1) + ×	162 colonies (1) + ×	Diffuse growth (1) — ×	
Diffuse (1) 5 colonies (2) — ×	Diffuse (1), (2) — ×	×	Growth diffuse in all plates at end of seventy-two hours.
×	×	×	Experiment repeated; no growth in any of the plates.
×	×	×	Colonies about equally distributed in all plates.
×	×	×	At end of forty-eight and sixty-eight hours growths more diffuse.
Diffuse (1), (2) — ×	×	×	At end of seventy-two hours growth increased in all plates.
×	×	Most colonies of all (1), (2) —	
Considerable growth (1) — ×	×	×	
×	×	×	
30 colonies (1) — ×	50 colonies (1) — ×	28 colonies (1) — ×	
×	×	×	
×	×	×	
×	×	×	Growth at end of ninety-six hours same: formaldehyd tests same. Involution form of bacterium used.
×	×	×	Distribution of colonies same in all plates. Fresh culture used.

to agar modified with ammonium hydroxid. The description of those marked +, formaldehyd present; —, formaldehyd absent.

COMMENT ON TABLE 3

Hexamethylenamin.—Bacterial growth was not prevented in any of these fluids even in solutions containing 0.5 per cent. of hexamethylenamin, a proportion much higher than could obtain anywhere in the body. It is therefore improbable that the administration of hexamethylenamin could have marked antiseptic effects in the tissues, although a partial restraining action is not positively excluded. Traces of formaldehyd were liberated, but not enough to be effective; i. e., it must have been less than 1:10,000.

Formaldehyd.—The limit of bactericidal efficiency in general is from 1:10,000 to 1:5,000 for a majority of the organisms. In the sterile ascitic fluid which was incubated with *B. typhosus,* bacteria and formaldehyd were simultaneously present in all concentrations, while in the experiment which consisted of ascitic fluid and *B. typhosus* no bacteria grew in any of the concentrations, although formaldehyd was present in all the cultures. No explanation can be offered for the results in these two experiments.

IV. Hexamethylenamin Itself is Not Bactericidal

The experiments just described indicate that the bactericidal efficiency of hexamethylenamin depends on the liberated formaldehyd. This was also the conclusion of Jordan,[9] who observed that the hexamethylenamin prevented putrefaction of urine and the growth of staphylococci when the formaldehyd concentration, as estimated by the phloroglucin test, corresponded to about 1:10,000; i. e., the same concentration in which pure formaldehyd is bactericidal. In alkaline urines putrefaction ensued more rapidly and staphylococci grew readily. This is indirect evidence that the antiseptic properties of hexamethylenamin must depend on the liberated formaldehyd. Others have made similar statements, but without offering decisive evidence that hexamethylenamin does not possess bactericidal properties.

The question was decided definitely in the negative by incubation experiments with several species of bacteria in hexamethylenamin media in which the liberation of formaldehyd was prevented. This medium consisted of agar modified with sodium carbonate, or with ammonium hydroxid. In this way an alkaline medium was secured which prevented the liberation of formaldehyd and allowed the hexamethylenamin to act as such on the bacteria.[10] All of the cultures consisted of plates. Different concentrations of hexamethylenamin were used, but the total volume of the culture in each case was always the same. The same volume of a

9. Jordan: Biochem. Jour., 1911, v, 274.

10. We wish to express our thanks to Professor Perkins, of the Laboratory of Hygiene and Preventive Medicine, who supplied us with pure cultures.

dilute culture of the bacteria was used in each plate. At the end of the incubation period, a small portion of the culture material was excised, treated with a little (5 c.c.) distilled water and tested with the phenylhydrazin and phloroglucin tests for formaldehyd.

The details of a typical experiment are as follows:

Experiment 9.—B. coli, bacterial emulsion consisting of three loops of a pure culture in 3 c.c. of bouillon; hexamethylenamin, 10 per cent. solution in water; ammonium hydroxid, 0.5 per cent. solution of the concentrated ammonium hydroxid (sp. gr. 0.9); and plain agar melted on a water-bath so as just to flow. The solutions were mixed in the order given in Table 4.

TABLE 4.—EXPERIMENT WITH COLON BACTERIA. RESULT*

Hexamethylenamin, c.c.	Concentration	Plain Agar† c.c.	Phloroglucin‡	Phenylhydrazin‡
1.0	1:50	8.3	+ sl	+ sl
0.5	1:100	8.8	—	—
0.2	1:250	9.1	—	—
0.1	1:500	9.2	—	—
0.0	:0	9.3	—	—

* Numerous colonies appeared at the end of twenty-four hours in all of the plates.

†Preceding the plain agar 0.5 c.c. ammonium hydroxid and 0.2 c.c. bacteria were added to the hexamethylenamin in the order named.

‡ Formaldehyd; + present; — absent; sl. = slight.

All plates were in duplicate. The mixing of the contents was accomplished by quickly and gently tilting and rotating each plate before the agar solidified. Then they were placed in an incubator at 37.5 C. At the end of twenty-four hours, and in some cases at the end of forty-eight hours, the plates were inspected and the formaldehyd tests applied.

The results of the experiments have been placed in Table 5.

COMMENT ON TABLE 5

The data from the first three experiments in which no alkali was added and in which liberation of formaldehyd took place, show that no bacterial growth occurred in concentrations of 1:100 and 1:50 of hexamethylenamin. In the lower concentrations bacteria grew about as effectively as in plates containing no hexamethylenamin. In these low concentrations of hexamethylenamin not enough formaldehyd was liberated to inhibit the growth of bacteria. The presence of formaldehyd in the plates was confirmed by the tests.

On the other hand, in agar modified with ammonium hydroxid and sodium carbonate, and which did not contain liberated formaldehyd, the growth of bacteria took place uniformly throughout all of the plates, even those containing 2 per cent. of hexamethylenamin. Usually the growths

were so diffuse that it was impossible to estimate the number of colonies present and they differed but slightly, if at all, from those of the blanks (consisting of the special agar minus hexamethylenamin). In a majority of the plates containing concentrations of 1:50 hexamethylenamin not quite enough alkali was present to inhibit completely the liberation of formaldehyd. Traces of formaldehyd could be detected, but not enough was present to affect the bacterial growth. The experiments with different species of organisms, some highly resistant and some susceptible to formaldehyd, yielded practically identical results. These results stand out as a convincing proof that hexamethylenamin in itself is not bactericidal, but that the bactericidal properties depend on the liberation of formaldehyd.

V. The Liberation of Formaldehyd from Hexamethylenamin Depends on the Hydrogen Ion Concentration of the Solution

Since the bactericidal properties of hexamethylenamin depend on the liberated formaldehyd, it is essential to ascertain the factors which determine this liberation under biological conditions. The possible factors are acidity, temperature, time, concentration, ferments and perhaps others. Of these, as has been previously remarked, the acidity of the fluid is of the greatest importance. Our results indicated that the liberation could occur with degrees of acidity lower than those appreciated by litmus, and closely approximating to that of certain body fluids. On the other hand, no decomposition whatsoever occurred in solutions rendered alkaline by 0.2 per cent. sodium carbonate, a degree of alkalinity frequently exceeded by certain body fluids, particularly in the gastrointestinal tract. Therefore, we resorted to the determination of the true reactions of fluids by the use of the indicater methods elaborated by Sörensen,[11] and later extended by L. J. Henderson[12] and others, to the various body fluids.

True Reaction.—Before passing on to the experiments themselves, it is necessary to say something concerning the principles on which our knowledge of the true reaction of a fluid is based. Briefly, the true reaction of a solution in terms of physical chemistry depends on the relative concentrations of the hydrogen (H) and hydroxyl (OH) ions therein. When the concentration of the hydrogen and hydroxyl ions are equal the condition is spoken of as neutrality. When there is a preponderance of hydrogen (H) ions true acidity exists, while a preponderance of hydroxyl (OH) ions means true alkalinity. The hydrogen may come from an acid such as hydrochloric acid (HCl) or an acid salt such as monosodium phosphate (NaH_2PO_4). In either case it is only the hydrogen which is ionized or dissociated that is pertinent to the acid reaction of a solution. These ions possess certain electric charges, for when water or electrolytes (salts) are decomposed electrolytically the acid ions aggregate at one pole, while the basic

11. Sörensen: Ergebnisse d. Physiol., 1912, xii, 394.
12. Henderson, L. J.: ibid., 1909, viii, 254; ibid., Jour. Biol. Chem., 1911, ix, 403.

ions aggregate at the other pole. Thus hydrogen ions carry a positive charge and aggregate at the negative pole. The mode of expression commonly used is $\overset{+}{(H)}$; for hydroxyl $\overset{-}{(OH.)}$

The reaction of a solution can be given a quantitative expression by measuring the values of $\overset{+}{(H)}$ and $\overset{-}{(OH)}$ (L. J. Henderson[13]). This is highly essential for the accurate study of the relation of reactions to different chemical phenomena. First, the point of neutrality must be known. It has been determined experimentally that at 25 C. the concentrations of $\overset{+}{(H)}$ is 0.000,000,1 gm., and that of $\overset{-}{(OH)}$ is 0.000,0017 gm. in 1,000 gm. of water. Dividing this by the atomic weights, it is seen that the number of $\overset{+}{(H)}$ and $\overset{-}{(OH)}$ ions is alike, as demanded by theory. Expressed logarithmically the figure for the ionized hydrogen $\overset{+}{(H)}$ would read $\overset{+}{H}^{10-7}$ as the quantitative expression of neutrality. For convenience the exponential digit alone without the minus sign is now used, that is, instead of $\overset{+}{(H}^{10-7})$, merely 7.0 is written. We have used this mode of expression in our paper. A number of actual $\overset{+}{(H)}$ determinations will serve to illustrate just what is meant by alkalinity and acidity:

Lowest $\overset{+}{(H)}$ concentration = 7.3		
7.2	true alkalinity.	
7.1		
7.0 = neutrality.		
6.9		
6.8		
6.7	true acidity.	
6.6		
Highest $\overset{+}{(H)}$ concentration = 6.5		

The method now commonly used for the determination of the $\overset{+}{(H)}$ concentration of fluids is that devised and described by Sörensen. The objections to this method raised by Rona[15] do not concern us here. The method of Sörensen consists essentially of a series of indicators which produce certain changes of color with certain definite range of the $\overset{+}{(H)}$ concentration. Sets of standards with known ranges of $\overset{+}{(H)}$ concentrations containing the different indicators are used for comparison with the unknown solution. In detail it consists of running 5 c.c. of the fluid, for instance, urine, in a bottle containing the indicator. This is then diluted to 125 c.c. with distilled water freshly boiled (to remove carbon dioxid) and previously cooled to a volume equal to that of the standard containing an equal volume of the indicator. The comparison is then made until a standard is found whose color exactly matches that of the unknown. The $\overset{+}{(H)}$ concentration of the matched standard is the $\overset{+}{(H)}$ concentration of the fluid. Certain precautions must be exercised in the selection of a practically neutral water for the dilution of fluids, and glassware must not be contaminated with bases or acids. Turbidity in the diluted solutions must be avoided. In such cases a smaller volume of fluid is used, as it has recently been

13. Henderson, L. J.: Science, 1913, xxxvii, 389.
15. Rona: Handbuch d. Biochemischen Arbeitsmethoden, 1911, v, 317.

shown by L. J. Henderson and Palmer[16] that dilution does not affect the determination of the $(\overset{+}{H})$ concentration to any appreciable extent.

Relation Between Formaldehyd Liberation and Hydrogen Ion Concentrations in Urines

We have examined[17] urines, cerebrospinal fluids, bloods, gastric juice and a few pathological exudates from persons receiving hexamethylenamin, and other fluids chiefly from dogs. In this section the discussion will be limited to human urines, since they exhibit the most variable concentrations of ionizable hydrogen $(\overset{+}{H})$. The remaining fluids will be taken up in the sections to follow.

The following tabulation is based on 292 hexamethylenamin urines, some freshly voided, others that were standing. (The influence of standing will be discussed later.) The freshly voided urines consisted of fractional specimens which were obtained as often as the individuals (dispensary patients[18] and laboratory assistants) could micturate. Standing urines represented collections of twenty-four hours in the majority of cases. These were obtained chiefly from patients in the wards. The dosage of hexamethylenamin varied, but enough was usually excreted in the urine so that liberation of formaldehyd could take place. The phloroglucin test for formaldehyd was used. The data obtained have been placed into Table 6. In this, the urines are arranged in the order of their $(\overset{+}{H})$ concentrations.

COMMENT ON TABLE 6

It is readily seen from Table 6 that free formaldehyd was present in the majority, or 221 (97.4 per cent.) of the urines which were acid; that is, $(\overset{+}{H})$ concentration over 7.0 (from 6.98 to 4.48). Of the six urines which were doubtful, three showed comparatively high acidities. This is ascribed to contamination of our receptacles, as judged from the reactions of the adjoining samples from the individuals, and from the fact that lower acidities generally showed the presence of formaldehyd. The remaining doubtful specimens possess acidities which are close to the neutral point, and these may have been contaminated or contain portions of alkaline fractions which reduced the intensity of the formaldehyd reactions.

Of 59 urines which exhibited alkalinity, that is $(\overset{+}{H})$ concentration of less than 7.0 (from 7.1 to 7.4), the majority or 57 (about 96.8 per

16. Henderson, L. J., and Palmer: Jour. Biol. Chem., 1913, xiii, 393.
17. The acidity determinations were made under the supervision of Professor Haskins of the Bio-Chemical Laboratory.
18. From the Medical Dispensary, Western Reserve University and Lakeside hospital.

cent.) show the absence of formaldehyd. The two urines which are marked doubtful may have contained portions of preceding acid urine fractions, and which would therefore contain traces of formaldehyd.

TABLE 6.—Relation of Hydrogen Ion Concentration of Hexamethylenamin Urines to Liberation of Formaldehyd

	$\overset{+}{(H)}$*	Total No. of Hexamethylenamin Urines with Formaldehyd			$\overset{+}{(H)}$*	Total No. of Hexamethylenamin Urines with Formaldehyd	
		Present	Absent			Present	Absent
^ Increasing alkalinity	7.4 ..	....	5	Increasing acidity ^	6.25 ..	2	....
	7.3 ..	....	8		6.24 ..	1	....
	7.25 ..	....	3		6.2 ..	9	....
	7.2 ..	....	29		6.15 ..	1	....
	7.16 ..	1	4		6.1 ..	5	....
	7.15 ..	....	2		6.0 ..	6	....
	7.1 ..	1(?)	6		5.95 ..	26	....
Neutrality—>	7.0 ..	1(?)	5		5.9 ..	2	....
	6.98 ..	....	1		5.85 ..	6	....
	6.95 ..	4	1		5.82 ..	1	....
	6.94 ..	....	1		5.8 ..	8	....
	6.92 ..	1(?)	....		5.75 ..	5	....
	6.9 ..	3	1		5.7 ..	3	....
	6.89 ..	1	....		5.65 ..	5	....
	6.85 ..	3	....		5.6 ..	4	....
	6.75 ..	2	....		5.58 ..	1	....
	6.72 ..	1	....		5.55 ..	1	....
	6.7 ..	10	1(?)		5.5 ..	3	....
	6.65 ..	1	....		5.47 ..	1	....
	6.64 ..	3	....		5.45 ..	1	....
	6.6 ..	7	....		5.4 ..	4	....
	6.55 ..	1	....		5.35 ..	1	....
	6.52 ..	1	....		5.3 ..	3	....
	6.5 ..	10	....		5.25 ..	1	....
	6.47 ..	4	....		5.2 ..	3	....
	6.45 ..	6	....		5.19 ..	6	....
	6.4 ..	1	....		5.1 ..	10	....
	6.38 ..	2	....		5.0 ..	16	1
	6.37 ..	1	....		4.85 ..	1	....
	6.35 ..	9	....		4.48 ..	1	....
	6.32 ..	1	....				
	6.3 ..	12	....		Total .	224	68

* $\overset{+}{(H)}$ refers to hydrogen ion concentration.

Of 6 urines which exhibited neutrality, that is, $\overset{+}{(H)}$ concentration of 7.0, the majority or 5 (83 per cent.) showed the absence of formaldehyd. Here again the single doubtful specimen may have contained a portion of previously used acid urine.

It was observed that many of the first, fractional urines (either acid or alkaline), which contained only a trace (more or less) of hexamethyl-

enamin as indicated by the bromin-water test, did not show detectable traces of formaldehyd. It is possible that not enough hexamethylenamin was present to liberate sufficient formaldehyd for the phloroglucin test. We have, therefore, omitted from the table the first fraction of urines voided.

CONCLUSIONS

Practically all hexamethylenamin urines which exhibit true acidity, that is, $(\overset{+}{H})$ concentration over 7.0, show the presence of formaldehyd; while urines which exhibit true alkalinity, that is, $(\overset{+}{H})$ concentration less than 7.0, do not show detectable traces of formaldehyd.[19] The very rare exceptions to this rule are probably technical errors.

VI. Previous Investigations on the Distribution, Excretion and Decomposition of Hexamethylenamin in the Body Fluids

It might be objected that the behavior of hexamethylenamin in body fluids *in vitro* under different conditions might be rather different than its behavior in the fluids of the living organism; and the question deserves to be investigated directly. In the preceding sections it was shown that the bactericidal properties of hexamethylenamin depend on the liberated formaldehyd, and that formaldehyd liberation can take place only in a truly acid medium. It is, therefore, important to determine directly whether hexamethylenamin is present in various tissues, fluids, etc., as such or as formaldehyd, and to note the true reaction of the various body fluids as well.

It has been shown by Crowe[20] that hexamethylenamin is excreted in the bile, pancreatic juice, cerebrospinal fluid and synovial fluid of human beings and in the saliva and milk of dogs; he supposed, but without any direct evidence, that it liberates formaldehyd in these situations. Flexner and Clark[21] and Hald[22] have reported its excretion in the cerebrospinal fluids of monkeys and patients ill with anterior poliomyelitis and meningitis, respectively. Bucura,[23] Rieder,[24] and Schmid and Schröter[25] have shown that free hexamethylenamin, but no free formaldehyd is excreted in human milk. Hanzlik[26] has shown that hexamethylenamin, but no free formaldehyd is excreted in human saliva. This has

19. During the course of our work a paper has appeared by G. W. Smith: Boston Med. and Surg. Jour., 1913, clxviii, 713, on a study of the $(\overset{+}{H})$ concentration in hexamethylenamin urines. Smith's results are practically identical with those obtained by us.

20. Crowe: Johns Hopkins Bull., 1908, xix, 109; ibid., xx, 102; Arch. intern. pharmacodyn., 1908, xviii, 315.

21. Flexner and Clark: Jour. Am. Med. Assn., 1911, lvi, 585.

22. Hald: Arch. f. exper. Path. u. Pharmakol., 1911, lxiv, 329.

23. Bucura: Arch. exper. Path. Therap., 1907, iv, 398.

24. Rieder: Monatschr. Kinderheilk., xi, 80; Chem. Abstracts, 1913, vii, 1057.

25. Schmid and Schröter: Cent. f. Physiol. u. Path. d. Stoffwechsel, 1910, v, 129.

26. Hanzlik: Jour. Am. Med. Assn., 1910, liv, 1940.

been confirmed by Zák,[27] who also detected hexamethylenamin in the bronchial secretion of a tuberculous patient; this has also been shown in sputa of tuberculosis and pneumonia by Armstrong and Goodman.[28] The elimination of hexamethylenamin in the middle ear has been shown by W. M. Barton;[29] in the anterior chamber of the eye by W. S. Gradle,[30] and in the aqueous and vitreous humors by Whitham.[31] In the urine the excretion of the drug as well as formaldehyd were first reported by Nicolaier.[32] Since then, however, considerable confusion has existed as to the constancy of the appearance of hexamethylenamin alone or in the liberation of formaldehyd. This confusion is reflected in the current text-books. Burnam[5] has reported that formaldehyd may be present or absent in either acid or alkaline urines. It has been reported by L'Esperance and Cabot[33] that only one-half of their patients excreted formaldehyd. These observers took no note of the reaction of the urines and used the phenylhydrazin test for formaldehyd. Jenness[34] reports that 53 per cent. of urines of two hundred individuals showed formaldehyd. Most of these were acid to litmus and the phenylhydrazin test was used. Using the phloroglucin test for formaldehyd, Sollmann[35] observed that only a small portion of the hexamethylenamin appears as formaldehyd if the urine is not voided too frequently. That acidity of urine facilitates formaldehyd liberation was suggested by Wiggs.[36] Jordan[9] reports that all acid hexamethylenamin urines (litmus and titration acidities) show the presence of formaldehyd by the phloroglucin test, and that alkaline urines never show it. These reports of inconstancy of liberation of formaldehyd in hexamethylenamin urines are due essentially to the lack of appreciation of a very important factor, namely, the true reaction of the urine. The time of sojourn of the urine in the bladder is very likely a contributory factor in facilitating the liberation of formaldehyd.

Concerning the presence of free formaldehyd in other body fluids after administration of hexamethylenamin, it has been heretofore shown by one of us that the saliva does not contain it and Rieder and Schmid and Schröter have shown that human milk does not contain formaldehyd. In other fluids such as bile, cerebrospinal fluid, blood serum, synovial, pericardial and pleural fluids, vitreous and aqueous humors no data are available from the literature as to the presence of free formaldehyd. This is due largely to the character of the tests employed. That is, these tests apply both to free formaldehyd and to formaldehyd liberated by the action of sulphuric acid (or other mineral acids) on hexamethylenamin.

VII. Hexamethylenamin Does Not Liberate Free Formaldehyd in Body Fluids, Except when Truly Acid, Namely, Urine and Gastric Juice

In the experiments to be described we have sought to determine whether hexamethylenamin exists as such or as free formaldehyd in the various body fluids and in freshly formed urine. This was investigated in experiments on dogs as well as in observations on fluids from human

27. Zák: Wien. klin. Wchnschr., 1912, xxv, 151.
28. Armstrong, J. J., and Goodman, E. H.: Jour. Am. Med. Assn., 1911, lvi, 1553.
29. Barton, W. M.: Jour. Am. Med. Assn., 1910, liv, 871.
30. Gradle, W. S.: Ophthalmic Record, March, 1911.
31. Whitham: Arch. Ophthalmology, 1912, xli, 604.
32. Nicolaier, A.: Ztschr. f. klin. Med., 1899, xxxviii, 350.
33. L'Esperance and Cabot: Boston Med. and Surg. Jour., clxvii, 577.
34. Jenness: Jour. Am. Med. Assn., 1913, lx, 662.
35. Sollmann: Jour. Am. Med. Assn., 1908, li, 818.
36. Wiggs: South. Med. Jour., Dec., 1910.

beings. The experiments on dogs consisted of injecting hexamethylenamin directly into a ligated loop of intestine and noting the time of the appearance of hexamethylenamin and formaldehyd in the blood and urine after short intervals of time.

All the animals received the regular laboratory anesthesia consisting of 0.02 gm. per kilo of morphin followed by ether. A tracheal cannula, a carotid cannula (for collecting samples of blood) and a vein cannula for saline injections were inserted. Laparatomy was performed; a long loop (40 cm.) of small intestine was exposed and injected with the hexamethylenamin dissolved in 100 c.c. of distilled water. From 25 to 50 c.c. of 0.9 per cent. saline were injected each time that a sample of blood was drawn in order to replace the fluid lost by repeated bleeding and to maintain diuresis throughout the experiment. The urine was collected by inserting a cannula into a ureter (usually the right) about 5 cm. above the point of entrance into the bladder. In Experiment 5 the ureteral cannula was placed as high as possible into the pelvis of the right kidney. In this way freshly formed urine was available for the tests.

Blood was collected from the left carotid artery at intervals of approximately five minutes. Each sample measured 10 to 15 c.c. This was rapidly defibrinated in a small evaporating dish, the clot of fibrin withdrawn and the remainder of the defibrinated blood was centrifugalized with a centrifuge possessing 3,000 revolutions per minute, which separates red blood-corpuscles from serum within five minutes, so that a perfectly clear and straw-colored supernatant fluid results. Approximately four minutes were required for defibrinating and five minutes for centrifugalization. Allowing one to two minutes for transfer of the serum, the entire process was completed in about ten minutes. The phloroglucin test was then applied directly to the serum. Inasmuch as the bromin-water test could not here be employed, a portion of the serum was reserved in each case, and this was distilled with a few drops of concentrated sulphuric acid and the phloroglucin test applied to the distillate.

The experiments were usually conducted until the height of hexamethylenamin excretion had been reached in the urine, as judged by the character of the precipitates with bromin-water. Then the animal was bled and finally killed with chloroform. The remaining body fluids were now obtained by carefully opening the different cavities and pipetting out the contents. Sufficient quantities of all fluids were usually obtained for the application of the tests, except cerebrospinal fluid. For free formaldehyd, the phloroglucin test was directly applied and the presence of hexamethylenamin was detected by distillation in the same manner as in blood-serum.

TABLE 7.—Results of Examination of Body Fluids in Experiment Four

Blood			Urine			Other Body Fluids (post-mortem)			
Time	Formaldehyd	Hexamethylenamin	Time	Formaldehyd	Hexamethylenamin	Fluid	Time	Formaldehyd	Hexamethylenamin
10:54	—	—	11:02	—	—	Pericardial	....	—	+
10:57	—	+*	11:07	—	+*	Pleural	....	—	....
11:12	—	+	11:15	+	+	Cerebrospinal	....	—	+
11:17	—	+†	11:20	+†	+†	Synovial	....	—	+
11:38	—	+†	11:26	+†	+†	Vitreous humor	....	—	+
11:54	—	+†	11:43	+†	+†	Aqueous humor	....	—	+*
12:26	—	+†	11:49	+	+	Bile	....	?	+
.....	....	....	12:06	+	+	Intestine contents	11:55	+	+‡
.....	....	....	12:32	+	+	Intestine contents	12:25	+	+‡

* Trace. † Marked. ‡ Strong. In this table, + = present; — = absent.

The results of the tests for formaldehyd in the fluids collected at different intervals were practically identical in all of the animals. A detailed protocol of one of the experiments (Table 7) is here appended to illustrate the mode of procedure.

Experiment 4.—Dog weighing 8.6 kg.; 0.2 gm. hexamethylenamin per kilo (total, 1.72 gm.) dissolved in 100 c.c. water and injected into ligated loop of intestine (50 cm.) at 10:54 a. m.

Bromin water did not give a precipitate with the dog's urine before the drug was given. Secretion of urine small during first part of experiment; perhaps due to withdrawal of considerable blood. Later secretion of urine improved by injections (50 c.c. at a time) of saline. Animal killed at 12:32 p. m.

The data from all of the experiments have been concentrated into Table 8.

TABLE 8.—LIBERATION OF FORMALDEHYD FROM

Animal Experiment Number	Hexamethylenamin, gm. per Kilo by Intestine	Blood			Urine		
		H F	Time of First Appearance After Injection (mins.)	Total Time Under Observation. (Mins.)	H F	Time of First Appearance After Injection (mins.)	Total Time Under Observation. (Mins.)
Cat 1 ..... 2.0 kg.	0.1	H + F —	5	25			
Dog 2 ..... 6.0 kg.	0.5	H + F —	.. ..	.. ..	H + F +	5 10	69 64
Dog 3 ..... 11.5 kg.	0.5	H + F —	5 ..	75 75	H + F +	5 10	83 78
Dog 4 ..... 8.6 kg. †	0.2	H + F —	3 ..	29 29	H + F +	13 21	25 17
Dog 5 ..... 5.6 kg. †	0.3	H + F —	4 ..	83 83	H + F +	12 26	75 61
Dog 6 ..... 8.0 kg.	0.3	H + F —	5 ..	85 85	H + F +	9 13	97 88
Average	....	H + F —	4.4 0	.. ..	H + F +	9 16	.. ..

† Flow of urine scanty.

* "H" refers to hexamethylenamin; "F" re fers to free formaldehyd; + means present;

COMMENT ON TABLE 8

Blood: The results as shown in Table 8 indicate that hexamethylenamin appears in blood about five minutes after administration of the drug. The maximum concentration appears in about thirty minutes. Of course, this will be influenced by the rate of absorption. It is shown that free formaldehyd did not appear in the bloods of any of the animals, and our protocols show that free formaldehyd was absent in all of the samples of

blood collected. The $(\overset{+}{H})$ concentration of blood-serum is known to be never below 7.0, and it may be slightly alkaline. The bloods of several dogs which we have examined showed it to be 7.0 as a rule, that is, it is neutral. This explains the absence of formaldehyd in bloods containing hexamethylenamin.

The bloods of fourteen different patients who received 2 gm. each of hexamethylenamin before the sample was collected, showed the presence of hexamethylenamin, but no free formaldehyd. The $(\overset{+}{H})$ concentrations of their serums ranged from 7.4 to 7.15, the mean value being 7.2.

HEXAMETHYLENAMIN IN BODY FLUIDS OF ANIMALS*

	Other Body Fluids					
Contents of Ligated Intestine	Cerebro-spinal Fluid	Pericardial Fluid	Pleural Fluid	Synovial Fluid	Aqueous Humor	Vitreous Humor
H + F +	H + F —	H + F —	H + F —	H + F —	H + F —	H + F —
H + F +	....	H + F —	H + F —	H + F —	H + F —	H + F —
H + F +	H + F —	H + F —	..... F —	H + F —	H + tr F —	H + tr F —
H + F —	H + F —	H + F —	..... F —	H + F —	H + F —	H + F —
H + F +	H + F —	H + F —	H + F —	H + F —	H + F —	H + F —
.....	.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	.....	.....	.....

— means absent; "tr" means trace.

Urine: Table 8 indicates that hexamethylenamin appeared in the urine of dogs in approximately nine minutes after administration. The time of maximum concentration, as judged by the character of the bromin-water precipitates, varied considerably in the different individuals; but in the animals with a good continuous secretion of urine it might be said to have taken place about forty-five minutes after administration.

The average time of the appearance of formaldehyd was sixteen minutes; that is, somewhat delayed as compared with that of hexamethylenamin. All of the urinary specimens collected were acid to litmus, which means that they possessed a fairly high degree of acidity by the indicator method used for the estimation of $(\overset{+}{H})$ concentrations. After formaldehyd made its appearance it was simultaneously present with hexamethylenamin in all of the freshly voided specimens of urine of each animal.

The greater portion of our data on urines was obtained from patients. Confined and ambulatory patients were used. In the case of the ambulatory patients, 2 gm. of hexamethylenamin were given by mouth, and the urine was collected as often as the patient could void it. The phloroglucin test for free formaldehyd was applied to the freshly voided specimens in the dispensary and the $(\overset{+}{H})$ concentrations were determined within two to three hours; the urines being meanwhile preserved by toluol. Patients that were confined in the wards received continuous and different doses of hexamethylenamin, their urines were collected (with toluol) for periods of twenty-four hours and the tests and the acidity determinations were performed at the end of this time. In some cases the urines stood for longer periods than twenty-four hours. The data on urines have been placed in two separate tables (Tables 9 and 10), and will be treated separately.

COMMENT ON TABLE 9

Freshly Voided Urines.—Table 9 indicates that free formaldehyd was present in practically all (98.9 per cent.) of the urines which were truly acid; that is, with $(\overset{+}{H})$ concentrations greater than 7.0. Formaldehyd was absent in the majority (98.1 per cent.) of urines which were truly alkaline; that is, those possessing $(\overset{+}{H})$ concentrations less than 7.0. Certain of the tests have been recorded as doubtful. This may be due either to a high dilution of the urines or to an insufficient amount of hexamethylenamin. In general, the intensity of the formaldehyd reaction was directly proportional to the $(\overset{+}{H})$ concentration of the urine; that is, the higher the acidity the more intense the red color, and the lower the acidity the less intense the color, so that oftentimes only traces of color could be detected. The individual protocols showed that hexamethylenamin was present in all of the fractional specimens voided.

The demonstration of free formaldehyd in the freshly voided urine, and even in the urine from the pelvis of the kidney in the dog experiments, shows that liberation must occur within the kidney, and that the formaldehyd effect must begin somewhere in the tubules. It is, therefore, possible for antiseptic action to occur at least in the pelvis of the kidney.

TABLE 9.—Relation of Hydrogen Ion Concentration to Liberation of Formaldehyd from Hexamethylenamin in Freshly Voided Urines

	Hydrogen Ion Concentration	Total No. of Hexamethylenamin Urines with Formaldehyd			Hydrogen Ion Concentration	Total No. of Hexamethylenamin Urines with Formaldehyd	
		Present	Absent			Present	Absent
^ Increasing alkalinity	7.4 ..	....	4	Increasing acidity v	6.15 ..	1	....
	7.3 ..	....	8		6.1 ..	5	....
	7.25 ..	....	2		6.0 ..	6	....
	7.2 ..	....	28		5.95 ..	26	....
	7.16 ..	....	3		5.9 ..	2	....
	7.15 ..	....	2		5.85 ..	6	....
	7.1 ..	1	6		5.82 ..	1	....
Neutrality—>	7.0 ..	1	4		5.8 ..	8	....
	6.98 ..	....	1		5.75 ..	5	....
v Increasing acidity	6.95 ..	2	1		5.7 ..	3	....
	6.92 ..	1	....		5.65 ..	5	....
	6.9 ..	2	....		5.6 ..	4	....
	6.85 ..	2	....		5.58 ..	1	....
	6.7 ..	9	....		5.55 ..	1	....
	6.65 ..	1	....		5.5 ..	3	....
	6.64 ..	2	....		5.47 ..	1	....
	6.6 ..	7	....		5.45 ..	1	....
	6.55 ..	1	....		5.4 ..	4	....
	6.52 ..	1	....		5.35 ..	1	....
	6.5 ..	9	....		5.3 ..	3	....
	6.47 ..	3	....		5.25 ..	1	....
	6.45 ..	4	....		5.2 ..	3	....
	6.38 ..	1	....		5.19 ..	6	....
	6.35 ..	4	....		5.1 ..	10	....
	6.3 ..	8	....		5.0 ..	16	1
	6.25 ..	2	....		4.85 ..	1	....
	6.24 ..	1	....		4.48 ..	1	....
	6.2 ..	9	....				
					Total .	196	60

COMMENT ON TABLE 10

Standing Urines.—It will be readily seen from Table 10 that practically identical results were obtained with old urines as with freshly voided specimens. That is, practically all of the urines which were truly acid showed the presence of formaldehyd, while those which were truly alkaline liberated no formaldehyd. It was stated that some of these urines stood longer than twenty-four hours. Among these were urines which were truly alkaline ($\overset{+}{H}$) concentrations less than 7.0, and after

standing two to three days they still showed no formaldehyd, although the quantity of hexamethylenamin present was abundant as was indicated by the character of the bromin-water precipitate. No formaldehyd, therefore, could be liberated in a bladder containing alkaline urine. It is conceivable, however, that in such cases formaldehyd liberated in the kidney might continue to act for a time in the bladder. This phase needs further investigation.

Cerebrospinal Fluid.—Table 8 shows that hexamethylenamin, but no free formaldehyd, appears in the cerebrospinal fluids of dogs. The quantities obtained were usually so small that not enough of the fluid was available for $(\overset{+}{H})$ concentration determinations.

TABLE 10.—Relation of Hydrogen Ion Concentration to Liberation of Formaldehyd from Hexamethylenamin in Standing Urines

	Hydrogen Ion Concentration	Total No. of Hexamethylenamin Urines with Formaldehyd			Hydrogen Ion Concentration	Total No. of Hexamethylenamin Urines with Formaldehyd	
		Present	Absent			Present	Absent
^ Increasing alkalinity	7.4 ..	....	1	v Increasing acidity	6.64 ..	1	....
	7.25 ..	....	1		6.5 ..	1	....
	7.2 ..	....	1		6.47 ..	1	....
	7.16 ..	1(?)	1		6.45 ..	2	....
Neutrality—>	7.0 ..	....	1		6.4 ..	1	....
v Increasing acidity	6.95 ..	2	....		6.38 ..	1	....
	6.94 ..	....	1		6.37 ..	1	....
	6.9 ..	1	1		6.35 ..	5	....
	6.89 ..	1(?)	....		6.32 ..	1	....
	6.85 ..	1	....		6.3 ..	4	....
	6.75 ..	2	....				
	6.72 ..	1	....		Total .	28	8
	6.7 ..	1	1(?)				

Observations as follows were made on patients.[37] Hexamethylenamin in different doses was given by mouth, and after a given interval of time, lumbar puncture was performed and the fluid was collected directly into a test-tube. The phloroglucin test for free formaldehyd was immediately applied directly to the fluid. The same mixture was then distilled after the addition of a little sulphuric acid and the same test was applied to the distillate. When positive this indicated the presence of hexamethylenamin. The data obtained have been placed in Table 11.

37. We wish to express our thanks to Drs. McClelland, Gammon, MacGregor, Lowe and Scott of the medical service of Lakeside hospital for collecting fluids from these patients.

TABLE 11.—Excretion of Hexamethylenamin and Formaldehyd in Cerebrospinal Fluid

Patient	Diagnosis	Character of Cerebrospinal Fluid	Formaldeyhd Present + Absent —	Hexamethylenamin Present + Absent —	Hydrogen Ion Concentration	Time of Lumbar Puncture After Administration of Drug, Hrs.	Quantity of Hexamethylenamin Given by Mouth, gr.	Number of Doses	Remarks
H.	Leprosy	Clear; colorless	—	—	...	¼	60	1	
Mrs. E.	Alcoholic neuritis	Clear; colorless	—	+*	7.1	½	60	1	$\overset{+}{\text{Blood, (H)}} = 7.2$
J. R.	Tabes	Clear; colorless	—	+	...	¾	60	1	
A. McM.	Cerebral arteriosclerosis	Clear; colorless	—	+	...	5/6	60	1	
S. S.	Epilepsy	Clear; colorless	—	+	...	1	60	1	
Mrs. S.	Cerebrospinal lues	Clear; colorless	—	+	7.3	1½	60	1	Had received 40 grains of boric acid
C.	Cerebrospinal lues	Clear; colorless	—	+	...	2	40	1	
F. Y.	Chronic gastritis	Clear; colorless	—	+	...	3	60	1	
M. V.	Pneumococcic meningitis	Amber; turbid; pneumococci present	—	+	...	18	180	9	Hexamethylenamin given; 20 gr. every two hours
M. V.	Pneumococcic meningitis	Amber; turbid; pneumococci present	—	+	...	84	840	42	Hexamethylenamin given; 20 gr. every two hours (continued)

* = Trace.

COMMENT ON TABLE 11

The results as shown in Table 11 indicate that hexamethylenamin appears in the cerebrospinal fluid about thirty to forty-five minutes after administration. This is practically confirmative of Hald,[19] who reported forty-five minutes. It is seen that no free formaldehyd appeared at any time in any of the fluids examined. The dosage varied from 40 grains (3.2 gm.) to 840 grains (54.5 gm.). Patient M. V., who had received 20 grains every two hours for fifty-one hours, whose total dosage amounted to 1,020 grains (66.2 gm.), and whose fluid was obtained on three consecutive days, showed no free formaldehyd at any time. At the end of this time there was marked vesical irritation with hematuria and the urine showed a high concentration of formaldehyd.

The $(\overset{+}{H})$ concentration was not obtainable in all of the fluids on account of their limited supply. Patients S. and E. showed $(\overset{+}{H})$ concentrations of 7.3 and 7.1, respectively. It is known that the $(\overset{+}{H})$ concentration of cerebrospinal fluid is about like that of serum. This is confirmed by the fluids of other patients which have been examined by us; that is, the tendency of the fluid is to be slightly alkaline or neutral. This is, undoubtedly, the reason why free formaldehyd is not liberated from hexamethylenamin in cerebrospinal fluid. The absence of formaldehyd indicates that there can be no antiseptic action in the spinal canal.

Other Body Fluids.—Table 8 shows that hexamethylenamin appears in the following fluids: pleural, pericardial, synovial, aqueous and vitreous humors. In no case could free formaldehyd be detected. Usually not enough of the individual fluids could be obtained for determinations of the $(\overset{+}{H})$ concentrations, but an examination of the fluids of two other healthy dogs showed that they are all neutral (7.0).

A child suffering with a tuberculous pleuritis was given one gram of hexamethylenamin before the chest puncture was made. Two hundred c.c. of a nearly colorless, but slightly turbid fluid were obtained, and this showed the presence of hexamethylenamin, but no free formaldehyd. The $(\overset{+}{H})$ concentration was 7.1. In other words, it was slightly alkaline and this explains why no free formaldehyd was liberated.

In April, 1911, the vaginal dressings[38] of four parturient women receiving hexamethylenamin were examined for the presence of free formaldehyd and hexamethylenamin. The lochia of these patients were light-straw colored, having been collected in each case about the tenth day of parturition. Two gm. of hexamethylenamin were given to each

38. We are indebted to Prof. Arthur H. Bill of the Department of Obstetrics for this material.

patient and the vaginal dressings were removed at the end of six to eight hours. These were then extracted with distilled water. The phloroglucin test for formaldehyd was then applied directly to the extracts and a portion was distilled with sulphuric acid. Hexamethylenamin was found present in each case, but no free formaldehyd.

Gastric juice, on account of its relatively high acidity, should liberate formaldehyd readily. Accordingly, we found both free formaldehyd and hexamethylenamin in stomach washings ($\overset{+}{H}$) = 4.0) of a patient receiving hexamethylenamin.

CONCLUSIONS

Following the administration of hexamethylenamin, the substance itself can be detected in all of the body fluids. Free formaldehyd does not appear in any of the body fluids which are neutral or truly alkaline. This includes blood, cerebrospinal, pleural, pericardial and synovial fluids, aqueous and vitreous humors, saliva, bile and some urines. Urines which are truly acid and contain hexamethylenamin practically always contain formaldehyd. Urine is the only body fluid, except gastric juice, which exhibits true acidity, hence, it is here only that liberation of formaldehyd from hexamethylenamin can take place.

VIII. Effect of the Administration of Monosodium Phosphate on the Reaction of Urine and Liberation of Formaldehyd

Since the acidity of urine depends on the presence of monosodium phosphate (NaH_2PO_4) and disodium phosphate (Na_2HPO_4), it would be interesting to know if the acidity of urine can be increased by the administration of the monophosphate. Its administration has been advised for this purpose. The acidity could conceivably be increased in two ways: 1. By increasing the concentration in the urine through an increased absorption of the phosphate. 2. By diminishing the relative concentration of the bases of the tissues at large, thereby simultaneously increasing the concentration of the monosodium phosphate which then would be excreted and increase its concentration in the urine. This is perhaps more probable because of the limited absorbability of the phosphate, as indicated by the experiment, a statement of which follows:

Subject P. J. H. took 13 gm. of monosodium phosphate at irregular intervals within three hours and forty minutes on a starvation day. The urines were collected in fractions and the acidities of the urines as well as the stools were determined according to the ($\overset{+}{H}$) concentrations. On previous occasions (two or three days preceding the experiment) the urine of P. J. H., while on a constant and mixed diet had been collected, and the ($\overset{+}{H}$) concentrations were determined in the fractional specimens throughout these periods. The urine possessed constantly a low acidity, that is, the average ($\overset{+}{H}$) concentration was about 6.8. Protocol of the phosphate experiment is given in full (Table 12).

During the day previous to the experiment he had received 2 gm. of hexamethylenamin. On the day of the experiment, breakfast consisting of 1 cup of coffee and 2 biscuits with butter was taken at 8 a. m.; no other food was taken during the day; about 200 c.c. of water was taken with each dose of the phosphate.

TABLE 12.—Result of Experiment with Monosodium Phosphate

Time		Hydrogen Ion Concentration	Hexamethylenamin	Formaldeyd	Remarks
8:30 a. m.	Passed stool, well-formed, same as usual	....	...	...	
10:35	Voided urine	6.45(?)	+	+	
11:00	Took 2 gm. NaH_2PO_4	....	...	...	
11:10	Voided urine	7.2	+	—	
11:20	Voided urine	6.7	+*	—	
11:40	Voided urine	6.55	—	—	
11:45	Took 2 gm. NaH_2PO_4	....	...	...	
12:03 p. m.	Voided urine	6.35	—	—	
12:05	Took 2 gm. hexamethylenamin	....	...	...	
12:15	Took 4 gm. NaH_2PO_4	....	...	...	
12:20	Voided urine	6.38	+*	—	
12:40	Voided urine	6.38	+†	+	Sensation of warmth in gastric region.
12:43	Took 1 gm. NaH_2PO_4	....	...	...	
1:05	Voided urine	6.30	+†	+†	Watery stool; suggestion of nausea.
1:35	Voided urine	5.70	+†	+†	Profuse watery stool; nausea.
1:40	Took 2 gm. NaH_2PO_4	....	...	...	
2:15	Voided urine	5.85	+†	+†	
2:18	Took 1 gm. NaH_2PO_4	....	...	...	
2:21	Profuse watery stool	....	...	...	
2:30	Voided urine	5.82	+	+	Profuse watery stool, clear almost like water; nausea.
2:40	Took 1 gm. NaH_2PO_4	....	...	...	
3:08	Took 0.5 gm. hexamethylenamin	....	...	...	
3:13	Voided urine	5.0	+	+	
3:40	Voided urine	4.85	+	+	Watery stool; light amber H—; F—; $\frac{+}{(H)} = 7.0$
4:25	Voided urine	5.40	+	+†	
4:38	Voided urine	5.75	+	+†	Desire to micturate after bladder is emptied.
4:40	Watery stool	7.0	+†	—	F, by phloroglucin direct; H, by distillation.
4:48	Voided urine	5.80	+†	+†	
4:52	Watery stool	7.0	+†	—	Tests same as at 4:40; 5:00 to 7:00 p. m., five watery stools.

* Trace. † Strong.

All of the stools, from 1:35 p. m. to 4:52 p. m., inclusive, gave marked white flocculent precipitates with magnesia mixture.

COMMENT ON TABLE 12

It is seen from Table 12 that the acidity of the urine gradually increased during the course of the administration of the phosphate, a result similar to certain ones obtained by L. J. Henderson and Palmer.[39] In the fore part of the experiment, the $(\overset{+}{H})$ concentration of the urine was very low, that is, 7.2 and 6.7. At the end of four hours and forty minutes after the administration of the salt was begun, the $(\overset{+}{H})$ concentration reached its maximum; that is, 4.85. This corresponded practically to the strength of a standard solution consisting of monosodium phosphate alone. In other words, at this time practically pure acid phosphate was being excreted in the urine. After this, the $(\overset{+}{H})$ concentration gradually began to diminish again, although the urine still remained markedly acid. Throughout the course of the experiment a more marked acidity of the urine was obtained than has been observed for some time in this subject. Two gm. of hexamethylenamin were taken during the course of the experiment. This appeared in the urine in fifteen minutes after administration and formaldehyd was present in all of the fractions which were truly acid. It was noticed that as the acidity increased the intensity of the formaldehyd reaction increased also.

During the experiment several watery stools were passed. The first stool appeared after 9 gm. of the salt had been taken, and within two hours and five minutes after the first dose was administered. In all, twelve stools appeared within six hours after the first stool. All of the stools were very thin and watery, straw-colored and contained a few shreds of mucus and presumably a little bacterial rest. On shaking, they frothed, and possessed an odor suggestive of fresh blood, consisting to some extent, perhaps, of serum. The $(\overset{+}{H})$ concentrations of each of three of the later specimens was 7.0; that is, the reaction was neutral, and on the addition of magnesia mixture they gave voluminous precipitates showing that they contained a large quantity of phosphate. The stools showed the presence of hexamethylenamin, but did not exhibit free formaldehyd.

This shows that the monosodium phosphate in its passage through the gastro-intestinal tract became neutralized and was to a large extent eliminated unabsorbed. It can be inferred from this that there was a depletion of bases of the blood and tissues at large to such an extent as to neutralize the acid phosphate. Further evidence of this exists in the fact that the acidity of the urine had been markedly increased. That is, the urine contained a high concentration of the acid monosodium phos-

39. Henderson, L. J., and Palmer: Jour. Biol. Chem., 1913, xiv, 81.

phate because of the impoverishment by the basic phosphate of the available sources for urine formation (blood and tissues at large). It is, therefore, possible to increase the true acidity of the urine at the expense of the bases of the blood and tissues at large.

Subject R. J. C. took 5 gm. of monosodium phosphate (1 gm. to a dose at intervals of a half hour) within three hours. One gm. of hexamethylenamin was taken with the first dose of the phosphate. On a previous day the urine had been rendered alkaline by taking sodium bicarbonate. The average $(\overset{+}{H})$ concentration of six fractional urines was 7.25 and hexamethylenamin was present in all specimens, but no free formaldehyd. The next three specimens obtained within the next three hours showed $(\overset{+}{H})$ concentrations of 6.6, 6.2 and 5.95, respectively. All of the specimens showed the presence of free formaldehyd. This experiment shows that the alkaline urinary fractions contained hexamethylenamin, but no free formaldehyd, and that as soon as the urine became truly acid, free formaldehyd was detectable. Presumably, the change in the reaction of the urine, that is, from alkalinity to acidity, was due to the monosodium phosphate.

TABLE 13.—EFFECT OF SODIUM BICARBONATE ON LIBERATION OF FORMALDEHYD

Time		Hydrogen Ion Concentration	Hexamethylenamin	Formaldehyd
11:00 a. m.	Voided urine	6.45	+*	+†
11:15	Voided urine	6.5	+*	+
11:20	Voided urine	6.52	+*	+
11:30	Voided urine	5.1?	+*	+
11:33	Took 1 gm. $NaHCO_3$	....	...	...
11:45	Took 1 gm. $NaHCO_3$	....	...	...
11:52	Voided urine	6.7	+*	+†
11:55	Took 1 gm. $NaHCO_3$	....	...	...
12:05 p. m.	Took 1 gm. $NaHCO_3$	....	...	...
12:20	Voided urine	6.92	+*	+†
12:40	Voided urine	7.0	+*	+?
12:58	Voided urine	7.2	+*	—
1:20	Voided urine	7.4	+*	—
1:50	Voided urine	7.4	+*	—
5:00	Voided urine	7.4	+	—
5:30	Voided urine	7.2	+	—

* Strong. † Trace. + = present. — = absent.

CONCLUSIONS

In an individual whose urine commonly shows a low degree of acidity it is possible to increase the acidity; that is, the $(\overset{+}{H})$ concentration, by the administration of monosodium phosphate. This gives rise to a profuse diarrhea and the stools contain the phosphate neutralized. Hexamethylenamin given by mouth at the same time appears unchanged in the stools, but formaldehyd is present in all fractions of urine which are

truly acid. The intensity of the formaldehyd reaction appears to be directly proportional to the acidity of the urine.

IX. The Administration of Alkali Renders the Urine Alkaline and Inhibits the Liberation of Formaldehyd from Hexamethylenamin

This was shown in the following manner. Sodium bicarbonate was administered by mouth during a course of hexamethylenamin medication. The urine was collected in fractions and the phloroglucin test was applied to the freshly voided urine. The reactions of the fractional urines were determined in the usual way. A protocol illustrative of the effect of sodium bicarbonate is here presented in detail.

Subject P. J. H., took 2 gm. of hexamethylenamin at 10:40 a. m. About 200 c.c. of water was taken at four different times.

For results, see Table 13.

A second experiment on another subject gave similar results.

COMMENT ON TABLE 13

It is seen from Table 13 that the administration of sodium bicarbonate diminishes the $(\overset{+}{H})$ concentration of the urine (also observed by L. J. Henderson and Palmer[39]), so that the urine becomes truly alkaline. Previous to the administration of the bicarbonate, several fractions of urine showed an average $(\overset{+}{H})$ concentration of 6.5. This was then reduced to a concentration of 7.4, which indicates true alkalinity. Hexamethylenamin and formaldehyd were present in all of the fractions which showed true acidity, but formaldehyd failed to appear when the urine became truly alkaline $(\overset{+}{H})$ concentrations of 7.2 and 7.4.

Six dispensary patients were given variable doses of potassium citrate together with hexamethylenamin. From some the urines were collected while the patients remained at the dispensary; others took the citrate over night and urines were collected the following day. In all, thirty-four fractional urines were collected, and the $(\overset{+}{H})$ concentration readings in the different patients ranged as follows: Patient V., 6.9 to 7.15; Patient J., 7.16 to 7.3; Patient K., 6.95 to 7.2; Patient F., 7.0 to 7.4; Patient P., 6.95 to 7.3; Patient D., 7.16 to 7.3. Hexamethylenamin was present in all of the freshly voided specimens, but no formaldehyd could be detected. The specimens were allowed to stand twenty-four hours and the phloroglucin test was again applied. In none of the specimens could any free formaldehyd be detected.

Disodium phosphate (Na_2HPO_4) and hexamethylenamin were administered to one subject on two different occasions; once, to another subject. Three gm. of the salt were taken each time and fractional

specimens of urine were collected as in previous experiments. The urines at all times were truly acid and contained hexamethylenamin and free formaldehyd.

CONCLUSIONS

The administration of sodium bicarbonate and potassium citrate alters the reaction of acid urine to true alkalinity, and the liberation of formaldehyd from such urines containing hexamethylenamin is inhibited.

X. The Rationale of Hexamethylenamin Therapy

From the foregoing correlation of chemical experiments with bacteriological and clinical data, it is clear that hexamethylenamin acts as an antiseptic only in proportion as it liberates formaldehyd, and that this liberation can only occur when the reaction is truly acid. Free formaldehyd is an efficient bactericide in comparatively high dilutions. Such an efficient concentration, no doubt, can be attained in normally acid urine. It is only in vesico-urinary conditions where we can expect any beneficial therapeutic action after the administration of hexamethylenamin, because urine is the only body fluid whose reaction may be altered, if necessary, to such an extent that liberation of formaldehyd from hexamethylenamin will be facilitated. This is not possible with other body fluids inasmuch as their neutrality or a slight tendency to alkalinity is rigidly maintained. No beneficial therapeutic responses, therefore, are to be expected in meningeal infections, infections of the cerebrospinal fluid or about the spinal cord, or of the infections of the ear, eye, synovial, pericardial or pleural fluids. We have not yet determined the position of bile in this respect. On account of the rapid absorption of hexamethylenamin and of the usual alkalinity of the bowel contents, no bactericidal action can be expected in the intestinal canal. It was shown in our experiments that the administration of monosodium phosphate (NaH_2PO_4) does not alter the reaction of the fluid from the intestines, and that the hexamethylenamin present under such conditions passes through the bowel unchanged. The administration of the monosodium phosphate increases the true acidity of the urine and the concentration of formaldehyd in such a urine containing hexamethylenamin is markedly increased. In the urine, therefore, an opportunity presents itself to increase the efficiency of hexamethylenamin therapy. This can be accomplished by the administration of the monosodium phosphate until the urine when tested with the phloroglucin reagent gives a reaction for formaldehyd. This test indicates quite closely the turning point in the reaction of urine. That is, at approximately the point of neutrality or beginning alkalinity it will no longer give a positive formaldehyd test with a hexamethylenamin urine, but as soon as such a urine begins to be truly acid it gives a positive test for formaldehyd. A urine which has been previously alka-

line, that is, possessing a hydrogen ion concentration of 7.2, can be rendered acid; that is, increased to a hydrogen concentration of 4.85 within approximately five hours after the administration of 13 gm. (200 grains) of the phosphate. If the occurrence of diarrhea is objectionable, the dosage of the phosphate may be reduced.

On the other hand, the administration of alkalies, such as sodium bicarbonate ($NaHCO_3$) or potassium citrate, entirely prevents the benefits of hexamethylenamin, and it is, therefore, irrational to prescribe them together. Our data were obtained largely from normal individuals, but so far as urine is concerned it would perhaps make little or no difference as to the behavior of hexamethylenamin in urines in various pathological conditions, since it is the reaction which is the essential factor in determining the decomposition. Recently, it has been shown by Henderson and Palmer[40] that urines in a great variety of pathological conditions are truly acid; that the tendency in many conditions is to a higher acidity than is normally found in urine of the average person. Such pathological urines would facilitate the liberation of formaldehyd from hexamethylenamin.

Concerning other body fluids our pathological data are as yet too scant to offer any suggestions in this direction. We have had thus far ten cerebrospinal fluids and one pleural exudate, and from the fact that they contained no free formaldehyd, it may be contended that no bactericidal action could have been expected. It is possible, if not probable, that the hydrogen ion concentration in certain other pathological fluids may be high enough to facilitate the liberation of formaldehyd from hexamethylenamin. With this end in view, the study of various pathological fluids is being continued.

We wish to express our thanks to Professor Sollmann for advice, and for criticism of the manuscript; to Professors Hoover and Phillips, and to Dr. Lester H. Taylor of the Medical Service for permission to use clinical material.

SUMMARY *

1. The phloroglucin test is the most delicate and most useful test for free formaldehyd.

2. Alkalies prevent, while acids facilitate the liberation of formaldehyd from hexamethylenamin in all body fluids.

3. The liberation of formaldehyd from hexamethylenamin in pathological fluids obeys the same laws as in other solutions; that is, it can only occur in acid reaction. Even when 0.5 per cent. hexamethylenamin was added to them, not enough formaldehyd was liberated to be bactericidal.

40. Henderson, L. J., and Palmer: Jour. Biol. Chem., 1913, xiii, 393.

* The numbers refer to the sections in the text.

4. Hexamethylenamin itself is not bactericidal.

5. Liberation of formaldehyd from hexamethylenamin depends on the excess hydrogen ion concentration of the solution above the neutral point.

6. Previous investigations leave us in doubt as to the behavior of hexamethylenamin in the body.

7. After administration, hexamethylenamin is present, but does not liberate free formaldehyd in blood, cerebrospinal, pleural, pericardial and synovial fluids, vitreous and aqueous humors, and urine when truly alkaline. Formaldehyd is liberated in urine which is truly acid, and in the acid gastric contents.

8. Administration of monosodium phosphate with hexamethylenamin renders the urine acid and facilitates the liberation of formaldehyd.

9. The administration of alkali with hexamethylenamin renders the urine alkaline and inhibits the liberation of formaldehyd.

10. The beneficial therapeutic effects of hexamethylenamin depend on the liberated formaldehyd. Such effects are to be expected principally, if not always, in acid urine only. It is irrational to prescribe alkalies (bicarbonate and citrate) together with hexamethylenamin.